Cultured monolayers of the dog jejunum with the structural and functional properties resembling the normal epithelium

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Weng, Xing-He, Klaus W. Beyenbach, and Andrea Quaroni. Cultured monolayers of the dog jejunum with the structural and functional properties resembling the normal epithelium. Am J Physiol Gastrointest Liver Physiol 288: G705–G717, 2005. First published November 18, 2004; doi:10.1152/ajpgi.00518.2003.—The development of a culture of the normal mammalian jejunum motivated this work. Isolated crypt cells of the dog jejunum were induced to form primary cultures on Snapwell filters. Up to seven subcultures were studied under the electron microscope and in Ussing chambers. Epithelial markers were identified by RT-PCR, Western blot, and immunofluorescent staining. Confluent monolayers exhibit a dense apical brush border, basolateral membrane infoldings, desmosomes, and tight junctions expressing zonula occludens-1, occludin-1, and claudin-3 and -4. In OptiMEM medium fortified with epidermal growth factor, hydrocortisone, and insulin, monolayer transepithelial voltage was −6.8 mV (apical side), transepithelial resistance was 1,050 Ω cm², and short-circuit current (Isc) was 8.1 μA cm². Transepidermal and paracellular resistances were estimated as 14.8 and 1.1 kΩ cm², respectively. Serosal ouabain reduced voltage and current toward zero, as did apical amiloride. The presence of mRNA of α-epithelial Na⁺ channel (ENaC) was confirmed. Na-D-glucose cotransport was identified with an antibody to Na⁺–glucose cotransporter (SGLT) 1. The unidirectional mucosa-to-serosa Na⁺ flux (19 nmol min⁻¹ cm⁻²) was two times as large as the reverse flux, and net transepithelial Na⁺ flux was nearly double the amiloride-sensitive Isc. In plain Ringer solution, the amiloride-sensitive Isc went toward zero. Under these conditions plus mucosal amiloride, serosal dibutyryl-cAMP elicited a Cl⁻-dependent Isc consistent with the stimulation of transepidermal Cl⁻ secretion. In conclusion, primary cultures of intestinal transport mechanisms and their regulation have been elucidated most clearly in cultured intestinal cells such as IEC-6 and IEC-18 derived from the normal small intestine of the rat (32, 33, 42) and the canine jejunal cell culture; epithelial sodium channel; sodium-glucose cotransporter 1; sodium absorption; chloride secretion

Our understanding of transport across the intestine rests largely on the study of intestinal segments perfused in vivo, excised segments in vitro, organ cultures, epithelial cell cultures, and, most recently, knockout animal models. The details of intestinal transport mechanisms and their regulation have been elucidated most clearly in cultured intestinal cells such as Caco-2 (1, 2, 21), T84 (27), HT-29 (24), COLO 205 (19), SW620 (52), and other epithelial cell lines derived from cancerous tissue. Only a few nontransformed intestinal epithelial cells (IEC) are available, such as IEC-6 and IEC-18 derived from the normal small intestine of the rat (32, 33, 42) and the IPEC-J2 cell line established from newborn piglet jejunum (44).

In the present study, we introduce a new primary culture of the normal jejunum with a focus on the method of producing that culture from crypt cells isolated from the dog jejunum. In addition, we provide a preliminary structural and functional characterization of the epithelial monolayer. Primary cultures and up to seven subcultures consistently formed confluent epithelial monolayers on Snapwell filters. The monolayers exhibit the morphological and functional polarization expected of the normal jejunum, including a prominent apical brush border. The tight junction presents claudin-3 and -4 as in the normal jejunum. The expression of epithelial Na⁺ channels (ENaC) and an amiloride-sensitive short-circuit current (Isc) can be attributed to the presence of culture-stimulating agents (epidermal growth factor, hydrocortisone, insulin), because this current disappears in plain Ringer solution lacking culture-stimulating agents. Fortuitously, the expression of an amiloride-sensitive Isc allows an estimate of the electrical resistance of transepidermal and paracellular pathways. The estimates reveal a leaky epithelium with a paracellular pathway 13 times as conductive as the transepidermal pathway. Measures of the unidirectional isotopic Na⁺ fluxes confirmed the leaky nature of the cultured monolayers and pointed to Na⁺ transport systems in addition to that mediated by ENaC. One such transporter is the Na⁺-D-glucose cotransporter SGLT1 identified by Western blot. When electrogenic Na⁺ absorption via ENaC is minimized by the use of plain Ringer solution containing mucosal amiloride, the addition of dibutyryl-cAMP (DBcAMP) to the serosal side activated a Cl⁻-dependent Isc consistent with the stimulation of transepidealtic Cl⁻ secretion. The culture can be studied for hours in Ussing chambers, thus affording detailed investigations of jejunal transport across a single layer of epithelial jejunal cells under well-defined experimental conditions.

Materials and methods

Epithelial cell isolation and culture. In general, the method to establish primary cultures from the small intestine of adult beagle dogs followed the procedures described for fetal human intestine (39). Segments of the small intestine were obtained from adult beagle dogs (1–2 yr old) that were killed for cardiac electrophysiological studies, as described by Fox et al. (14) and approved by the Center for Research Animal Resources at Cornell University. Segments of the jejunum (30–50 cm long) were removed and placed on ice. The lumen was washed four times with 50 ml ice-cold sterile PBS (in mM: 136.9 NaCl, 2.7 KCl, 1.5 KH2PO4, and 6.5 Na2HPO4), filled with a 1.25% trypsin-0.5 mM EDTA solution, clamped at the two ends, and incubated at room temperature for 10 min. The segments were then cut open longitudinally and laid flat on a sterile glass plate on ice, and the apical surface was scraped gently with a razor blade to remove mucus.
and most of the villi. The scrapings were discarded. Harder scraping, leaving behind only serosal layers, yielded intact crypts embedded in fragments of surrounding tissue that were collected in 50-ml centrifuge tubes containing serum-free OptiMEM (Invitrogen-GIBCO, Carlsbad, CA) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (antibiotic-antimycotic solution; Invitrogen-GIBCO-BRL). After centrifugation at 1,000 rpm (220 g), the supernatant was discarded. The pellet was washed three times with serum-free OptiMEM and then incubated with serum-free OptiMEM containing 0.4 mg/ml collagenase type IV (filter sterilized; Sigma, St. Louis, MO) at 37°C for 45–60 min, with gentle and brief shaking every 10–15 min. The dissociated epithelial cells were spun down at 1,000 rpm (220 g) and washed five times with 40 ml of 10% FBS (Hyclone Laboratories, Logan, UT) in DMEM supplemented with antibiotic-antimycotic solution. After each centrifugation at 1,000 rpm, the pellet was resuspended by pipetting up and down vigorously using a 10-ml wide-bore Falcon pipette (Milan, Gaithersburg, MD). The final pellet was suspended in 100–250 ml “fortified OptiMEM” (vide infra). After large fragments were allowed to sediment within 1–2 min, the upper two-thirds of the suspension were plated in 100-mm-diameter dishes coated with extracellular matrix ECL (Upstate Biotechnology, Lake Placid, NY) and incubated at 37°C and 6% CO2.

For the culture of monolayers and for their initial physiological characterization, we used fortified OptiMEM, which we define as serum-free OptiMEM supplemented with 10 mM HEPES, pH 6.5, 2.5 mM glutamine, 50 μM penicillin, 50 μg/ml streptomycin, 2 mM GlutaMAX-I (Invitrogen-GIBCO-BRL), 20 ng/ml epidermal growth factor (human recombinant; Upstate Biotechnology), 150 mM hydrocortisone 21-hemisuccinate sodium salt (Sigma), 10 μg/ml insulin (human recombinant; Sigma), and 4% FBS. After 3 h, fortified OptiMEM was aspirated, and the attached cells (consisting almost exclusively of intact or large portions of crypts) were rinsed four times, refed with fortified OptiMEM, and incubated at 37°C. After 24 h, the incubator temperature was changed to 32–34°C for the rest of the culture period.

After the primary cultures had grown in 100-mm-diameter dishes, between 300,000 and 400,000 cells in 0.5 ml fortified OptiMEM were subcultured onto Snapwell permeable filters (insert growth area 1.13 cm², 0.4 μm pore size; Costar, Cambridge, MA) and incubated at 32°C and 6% CO2 to form dog intestinal epithelial cell (DIEC) monolayers.

We evaluated the growth of DIEC monolayers by visual inspection under the microscope (Nikon Diaphot) and by daily measurements of the resistance across the filter area with a hand-held Millicell-ERS volt- and ohmmeter (Millipore, Billerica, MA). Because this resistance measurement (between two points with heterogeneous current distribution) is meant to monitor culture growth to confluence, we call this resistance “growth area resistance” (see Fig. 4e). For measurements of the transepithelial resistance (Rt) under more heterogeneous conditions of transepithelial current distribution, we used current-voltage (I-V) plots measured across confluent monolayers in the Ussing chamber.

**Immunocytochemistry.** Cells of primary cultures or passages 1 to 3 were washed three times with PBS, fixed with 3% formaldehyde, and then directly processed for immunofluorescence staining or permeabilized by one of the following two methods: 1) incubation with acetone-methanol 1:1 at −20°C for 10 min; or 2) lysis with 0.2% Triton X-100 in PBS for 2 min at room temperature. Further processing steps are described by Tian and Quaroni (49). The secondary antibodies were FITC- or rhodamine-conjugated goat anti-mouse or -rabbit anti-rabbit IgG (Boehringer Manheim, Indianapolis, IN) diluted 1:25 in PBS plus 2% BSA. Cells were counterstained with 0.01% Evans blue and 2 μg/ml DAPI for 2 min. After incubation with antibody and washing, the cells were mounted in glycerol-PBS (9:1) and 2.5% 1,4-diazabicyclo[2.2.2]octane and covered with coverslips.

Stained cells were examined with a Zeiss Axiosvert 10 microscope equipped with epifluorescence optics and an Olympus three-chip CCD camera. Digital images were processed with Adobe Photoshop software. We used the following primary antibodies: 1) CY-90 (Sigma) against keratin 18, mouse monoclonal, diluted 1:400; 2) anti-desmosomal protein, mouse monoclonal (Sigma), diluted 1:400; 3) anti-human occludin, mouse monoclonal, OC-3F10 (Zymed catalog no. 33–1500; Zymed), diluted 1:1,000; 4) anti-claudin-1, rabbit polyclonal (JAY.8, catalog no. 51–9000; Zymed), diluted 1:1,000; 5) anti-claudin-2, rabbit polyclonal (MH44, catalog no. 51–6100; Zymed), diluted 1:1,000; 6) anti-claudin-3, rabbit polyclonal (Z23.JM, catalog no. 34–1700; Zymed), diluted 1:1,000; 7) anti-claudin-4, mouse monoclonal (3E2C1, catalog no. 32–9400; Zymed), diluted 1:2,000; 8) anti-claudin-5, rabbit polyclonal (rabbit Z43.JK, catalog no. 35–2500; Zymed), diluted 1:1,000; and 9) anti-claudin-5, mouse monoclonal (4C3C2, catalog no. 34–1600; Zymed), diluted 1:2,000.

Cells were processed for transmission and scanning electron microscopy as previously described (41).

**Western blot.** Total cell lysates solubilized in SDS-PAGE sample buffer were subjected to SDS-PAGE and Western blot as described previously (49). The DNA concentration was determined using the Hoechst-33258 DNA assay and a mini-fluorometer (Hoefer, Pharmacia Biotech, Piscatway, NJ). The amount of cell lysate applied to each well was normalized to DNA (39). Lysates obtained from 0.2 × 10⁶ cells/sample were subjected to SDS-PAGE on a 7.5–10% acrylamide gel. The proteins were transferred to a nitrocellulose membrane (High-bond nitrocellulose; Amersham Life Science, Arlington Heights, IL) using a transblot system (Bio-Rad, Hercules, CA) at 100 V and 5°C for 90 min. The membranes were then blocked in 80 mM Na2HPO4, 20 mM NaH2PO4·2H2O, 100 mM NaCl, and 0.1% Tween 20 containing 3% BSA at 4°C overnight. Incubation with antibodies, washing, protein detection, antibody stripping, and reprobing were performed according to the ECL-Plus Western blotting protocol from Amersham Life Science. Blots were scanned with a Molecular Dynamics Storm 840 scanner (Amersham Pharmacia Biotech, Sunnyvale, CA) in the fluorescence mode. In other experiments, we employed fluorescent secondary antibodies and the Li-COR Odyssey Infrared Imaging System (Lincoln, NE). After transfer of the proteins to a Hybond-P/polyvinylidene difluoride (PVDF) membrane (Amersham Pharmacia Biotech) and blocking for 4 h in Odyssey Blocker, incubation with primary antibodies was done in Odyssey Blocker containing 0.1% Tween 20 overnight at 4°C. After four washes with Tris-buffered saline (TBS: 20 mM Tris base, 137 mM NaCl, pH 7.4) containing 0.1% Tween 20, the blots were incubated with Alexa Fluor 680 goat anti-mouse IgG (1:2,500 dilution in Odyssey Blocker, 0.1% Tween 20) for 1 h at room temperature and shielded from light. After being washed, the blots were scanned in the Odyssey Infrared scanner. The primary antibodies used are the same as listed above.

In a separate experiment, the presence of SGLT1 in DIEC cell lysates was examined by Western blot with an antibody specific to rabbit SGLT1. In these studies, SDS-PAGE and the transfer of protein from gel to PVDF membrane was done as described previously (55). After the membrane was incubated for 2 h in blocking solution containing 3% BSA, the membranes were then blocked in 80 mM Na2HPO4, 20 mM NaH2PO4·2H2O, 100 mM NaCl, and 0.1% Tween 20 containing 3% BSA at 4°C overnight. The antibody 8821 was first preincubated with 0.5 μg/ml of the immunizing peptide for 2 h, which yielded a negative Western blot. Both antibody 8821 and immunizing peptide were gifts of Dr. E. M. Wright (University of California Los Angeles). The antibody 8821 has been used successfully in the past in investigations of SGLT1 in the dog jejunum in vivo (22).

**Electrophysiological studies in Ussing chambers.** Electrophysiological measurements were performed on DIEC monolayers mounted in Ussing chambers (CHM5; World Precision Instruments, Sarasota, FL). Monolayers of passage 1 through 7 were studied. Finding consistent electrophysiological properties among them, we did most experiments on passages 1 to 3.
In most transport experiments, fortified OptiMEM (10 ml) was present on both sides of the epithelium. Using the methods of atomic absorption spectrophotometry, flame photometry, and coulometry, we measured the following partial composition of the OptiMEM culture medium: Na⁺ concentration, 157.0 ± 3.7 mM (n = 3); K⁺ concentration, 4.6 ± 0.3 mM (n = 3); Cl⁻ concentration, 123.7 ± 4.7 mM (n = 3); and protein, 0.56 ± 0.01 mg/ml. According to the manufacturer of OptiMEM culture medium, the glucose concentration is 13.9 mM (personal communication); other ingredients of OptiMEM culture medium are proprietary information.

In experiments examining the ion dependence of the measured Iₑ, confluent monolayers were taken from their cultures in fortified OptiMEM and mounted in plain Ringer solution lacking culture-medium ingredients, 4.6 mM Na⁺, 4.1 mM K⁺, and 157.0 mM Cl⁻. Using the methods of atomic absorption spectrophotometry and flame photometry, we measured the following partial composition of the OptiMEM culture medium: Na⁺ concentration, 4.6 ± 0.3 mM (n = 3); Cl⁻ concentration, 123.7 ± 4.7 mM (n = 3); and protein, 0.56 ± 0.01 mg/ml. According to the manufacturer of OptiMEM culture medium, the glucose concentration is 13.9 mM (personal communication); other ingredients of OptiMEM culture medium are proprietary information.

In the present study, we produced 17 primary cultures of DIEC. Some primary cultures were taken to seven passages before they lost proliferative activity. As long as the cells could be subcultured, they retained the ability to form confluent monolayers with similar morphological and electrophysiological properties.

### RESULTS

#### Cell proliferation and differentiation in primary and secondary cultures.

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#### Table 1. Effects of amiloride on unidirectional Na⁺ fluxes and Iₑ

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Jₑ$_{un}$ nmol/min cm⁻²</th>
<th>Jₑ$_{un}$ nmol/min cm⁻²</th>
<th>Jₑsc (cm⁻²) nmol/min cm⁻²</th>
<th>As current μA/cm²</th>
<th>Iₑ, μA/cm²</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>18.6 ± 2.2 (6)</td>
<td>9.7 ± 2.6 (6)</td>
<td>8.9</td>
<td>14.3</td>
<td>8.7 ± 1.1 (12)</td>
</tr>
<tr>
<td>Mucosal amiloride (10 μM)</td>
<td>18.9 ± 2.6 (6)</td>
<td>11.6 ± 1.7 (6)</td>
<td>7.3</td>
<td>11.7</td>
<td>1.1 ± 0.2 (12)</td>
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Values are means ± SE; nos. in parentheses are no. of dog intestinal epithelial cell monolayers. Jₑ$_{un}$ and Jₑ$_{un}$ are, respectively, isotopic Na⁺ fluxes from mucosal to serosal and from serosal to mucosal solutions; Jₑsc (cm⁻²), net mucosal-to-serosal flux; Iₑ, short-circuit current. *Significantly different (P < 0.001) from control by Student’s t-test. †Significantly different (P < 0.001) from control by Student’s t-test.
Although the culture steps producing DIEC are straightforward and reliable, a few steps proved particularly important. First, it was crucial to start cultures with intact or nearly intact crypts because isolated epithelial cells or fully differentiated villus cells did not attach to the substrate, nor did they survive for more than 2–3 days. As shown in Fig. 1a, essentially intact crypt cells attach to the substrate after only 4 h of incubation, and cells at the border of the cluster start to spread out and migrate. The use of ECL-coated dishes significantly improved (2- to 3-fold) the number of crypt cells attaching to substrate, but ECL-coated dishes were not essential, since qualitatively similar results were obtained with uncoated dishes. Nonepithelial cells, large tissue fragments, and clumps of villus cells did not adhere strongly and were washed out. Proliferative (mitotic) cells could be detected by staining mitotic apparatuses with anti-tubulin antibodies after only one night of incubation. However, mitotic apparatuses were relatively rare and flat and therefore difficult to detect by direct examination of unstained cultures.

Figure 1b shows that the crypt cells had spread out entirely to occupy a much larger surface after 1 day. The staining of these cultures with anti-keratin antibodies confirmed the epithelial nature of all, or nearly all cells (99.9% in 12 samples) present in these primary cultures (Fig. 1c). Contamination by fibroblasts and other cell types was negligible. Staining with a monoclonal antibody (TS23) specific for a glycosylated form of Notch-1 expressed in human and rat intestinal stem cells (Quaroni and Beaulieu, unpublished observation) indicated the presence of a subpopulation of stem cells in the primary cultures (Fig. 1d).

A second crucial step proved to be temperature control. When cultures were maintained at 37°C, they remained viable for at least 3–4 wk, but cell proliferation ceased entirely after 1 wk. In contrast, when cultures were grown at 32–34°C starting on day 2, regions containing rapidly proliferative cells became evident after 3–4 days (Fig. 1e). Proliferative cells incorporated bromodeoxyuridine, demonstrating rapid cell division and expansion in the cell population (Fig. 1f). Older, nonproliferative cells became enlarged and apoptotic after ~2 wk. By that time, the smaller proliferative cells represented the main cell type that could also be serially passaged. We have estimated a population doubling time to be 18–27 h in nine cultures. Proliferative cells could be readily subcultured at least six times, yielding epithelial cells that were characterized and used in all subsequent studies. We have called these cells DIEC.

When confluent, DIEC maintained a typical epithelial morphology and formed tightly confluent DIEC monolayers with frequent, obvious domes when grown on solid (plastic) sup-

Fig. 1. Primary cultures of dog intestinal (jejunal) epithelial cells (DIEC) grown in fortified OptiMEM. a: Crypt cells attach to the ECL-coated dish 4 h after seeding and wash out of nonattached cells; b: same culture as in a 1 day later. Epithelial cells have now attached and spread out without a significant increase in the number of cells; c: 1-day-old culture stained with an immunofluorescent antibody to keratin, demonstrating the epithelial nature of the culture; d: 1-day-old culture stained with a monoclonal antibody (TS23) specific to the glycosylated form of Notch-1, identifying crypt stem cells; e: 7-day-old culture maintained at 35°C, showing the presence of clusters of small proliferative cells (*) among older and larger nonproliferative cells; f: same culture as in e incubated with bromodeoxyuridine (BrdU) for 24 h to visualize proliferative cells (green-blue). BrdU incorporated into cellular DNA was visualized by immunofluorescence staining. Bar = 25 μm in a-f.
port, indicating transepithelial ion and water transport in the
direction of absorption. The presence of tight junctions could
be readily demonstrated by microscopic studies and by West-
ern blotting with antibodies to junctional proteins. As shown in
Fig. 2a, of the five claudins examined, only claudin-3 and -4
were detected in primary, first-, and second-passage cultures.
In some experiments, small amounts of claudin-1 were also
seen. Another tight junction protein, occludin-1, could also be
identified by Western blot (Fig. 2a). Claudin-2 and claudin-5
were not detected in any primary culture or passage. In these
experiments, β-tubulin was used as a loading control (Fig. 2a).

Immunostaining of primary or secondary cultures localized
the expressed junctional proteins to the paracellular pathway.
Occludin-1 appeared in a discrete pericellular band as expected
from its association with tight junctions (Fig. 2b). Likewise,
zonula occludens (ZO)-1 was distributed in a pattern consistent
with its presence in tight junctions (Fig. 2c). The distribution
of claudin-3 and claudin-4 was more diffuse than that of occludin
and ZO-1. Claudin-4 and claudin-3 (data not shown) appeared
to expand well beyond tight junctions (Fig. 2d). The distribu-
tion of desmosomes outlines again the paracellular space of
confluent epithelial cells (Fig. 2e).

Electron microscopy. When DIEC were cultured in plastic
dishes in the presence of fortified OptiMEM, both primary
cultures and subcultures formed monolayers, with the baso-
lateral membrane adhering to the substrate and microvilli on
the apical membrane facing the culture medium (Fig. 3a).
Next to this polarization, transmission electron micrographs
revealed extensive infoldings of the basolateral membrane
(Fig. 3, b–e), tight junctions near the apical border (Fig. 3d),
and desmosomes near the serosal border (Fig. 3e). Some
cells of the DIEC monolayers contain granules in the apical
cytoplasm, possibly identifying primitive goblet cells (Fig.
3, a–c). Our attempt to identify goblet cells with antibodies
to mucin did not succeed, since the rat and human antibodies
apparently did not recognize mucin in the dog. Cells without

Fig. 2. Expression of paracellular proteins in dog
intestinal epithelial cells (DIEC) grown in fortified
OptiMEM. a: Claudins-3 and -4 and occludin-1
were detected by Western blot in cell lysates
derived from primary cultures (P) and 1st and 2nd
passages of DIEC; b: occludin-1 (green); the arrow
points to a cell undergoing mitosis; c: zonula
occludens (ZO)-1 (green); d: claudin-4 (green); e:
desmosomes (green). The tight junction proteins
occludin-1, ZO-1, and claudin-4 were visualized
in DIEC of the 2nd passage. Bar = 25 μm in b–e.
granules are probably epithelial cells with the properties of transepithelial transport.

Scanning electron micrographs of primary and secondary cultures grown on plastic support for 10 days revealed epithelial cells with sparse apical microvilli (Fig. 4, a and b). Adjacent epithelial cells overlap, hiding their tight junctions below their lateral edges. In contrast, cultures grown on Millicell HA filters or Snapwells yielded taller cells with a prominent brush border (Fig. 4, c and d). DIEC monolayers of this type were used in the transport studies described below.

Daily measurements of the electrical resistance across the insert growth area revealed an exponential rise in resistance as DIEC monolayers became confluent (Fig. 4e). After 3–4 days, the electrical resistance increased sharply, reaching peak resistances 6–7 days after seeding the culture. Thereafter, resistance decreased together with increasing variability. In view of the transient nature of the $R_t$, we confined our study on transepithelial transport in Ussing chambers to monolayers between 4 and 6 days old.

Electrophysiology of DIEC monolayers in fortified Opti-MEM culture medium. Primary cultures of DIEC were not studied. Instead, our electrophysiological observations focused largely on confluent DIEC monolayers of the first, second, and third passage after noting no major differences with subsequent passages. Monolayers showing signs of apoptosis or degeneration were not used in electrophysiological studies. Under control conditions in fortified OptiMEM containing culture-stimulating agents (epidermal growth factor, hydrocortisone, and insulin), the DIEC monolayers had an open-circuit voltage ($V_i$) of $-6.8 \pm 0.6$ mV ($n = 36$) with a range from $-2.0$ to $-14.4$ mV, apical side negative. Apical side positive voltages were never observed. $R_t$ was $1,050 \pm 105 \Omega \cdot \text{cm}^2$ on average with a range from 429 to 2,173 $\Omega \cdot \text{cm}^2$ ($n = 22$). When DIEC monolayers were voltage clamped at 0 mV, $I_{sc}$ was $8.1 \pm 0.4 \mu A/\text{cm}^2$ ($n = 36$), with positive current flowing from the apical to the basolateral side. The $I_{sc}$ ranged from 5.3 to 13.3 $\mu A/\text{cm}^2$.

Amiloride-sensitive $I_{sc}$ and expression of ENaC in DIEC monolayers grown and studied in fortified Opti-MEM culture medium. The effects of amiloride were studied in the presence of fortified OptiMEM (Fig. 5). The control $V_i$ was $-3.6 \pm 0.4$ mV ($n = 10$), the $R_t$ was $694 \pm 72 \Omega \cdot \text{cm}^2$ ($n = 7$), and the $I_{sc}$ was $8.4 \pm 0.6 \mu A/\text{cm}^2$ ($n = 10$; Fig. 5A). After amiloride (10 $\mu M$) was added to the apical side, $V_i$ dropped immediately and significantly to $-0.7 \pm 0.2$ mV ($P < 0.001$), and $I_{sc}$ significantly decreased to $1.3 \pm 0.4 \mu A/\text{cm}^2$ ($P < 0.001$, Fig. 5A).

The inhibitory effects of amiloride were immediate, requiring 1–2 s to reach full effect. Even though amiloride significantly inhibited the $V_i$ and $I_{sc}$, it had no significant effect on $R_t$, which was $694 \pm 72 \Omega \cdot \text{cm}^2$ under control conditions and $687 \pm 62 \Omega \cdot \text{cm}^2$ ($n = 7$) in the presence of amiloride (Fig. 5A).

A consistent observation was the subsequent decrease of $R_t$ in the presence of amiloride. After amiloride had inhibited $V_i$, $R_t$ decreased together with increasing variability. In view of the transient nature of the $R_t$, we confined our study on transepithelial transport in Ussing chambers to monolayers between 4 and 6 days old.
and $I_{sc}$ within 1–2 s of application, these two variables remained unchanged in the presence of amiloride. In contrast, $R_t$ began to decrease gradually yet significantly, dropping to 81.3 ± 4.3% ($P < 0.001$) of control. The nature of this resistance drop was beyond the scope of the preliminary electrophysiological characterization of DIEC monolayers.

Amiloride (10 $M$) added to the basolateral solution had no significant effect on $V_t$, $R_t$, and $I_{sc}$ (Fig. 5B). In these nine DIEC monolayers, the control $V_t$ was 6.6 ± 0.8 mV, the $R_t$ was 887 ± 82 $\Omega \cdot$cm$^2$, and the $I_{sc}$ was 7.2 ± 0.3 $\mu$A/cm$^2$. After amiloride was added to the serosal side, $V_t$ decreased to −5.1 ± 0.6 mV, $R_t$ decreased to 731 ± 80 $\Omega \cdot$cm$^2$, and $I_{sc}$ decreased to 7.0 ± 0.3 $\mu$A/cm$^2$. None of these changes was significant.

We also examined the effect of amiloride on $V_t$, $I_{sc}$, and $R_t$ at 32°C, the temperature at which monolayers were cultured. At this temperature, 10 $\mu$M amiloride added to the apical side of the monolayer inhibited $V_t$ and $I_{sc}$, again with no effects on $R_t$ (data not shown).

Figure 6A shows the linear $I$-$V$ plot measured across DIEC monolayers in the absence and presence of amiloride. The slope of the $I$-$V$ plots is the transepithelial conductance ($1/R_t$), and the y-intercept is the $I_{sc}$. Apical amiloride (10 $M$) significantly reduced the $I_{sc}$ from 7.5 ± 0.3 to 0.9 ± 0.2 $\mu$A/cm$^2$ ($P < 0.001$), without affecting transepithelial conductance (1.49 and 1.42 mS/cm$^2$ before and after amiloride, respectively). Significant effects on $V_t$ and $I_{sc}$ but not on $R_t$ suggest that the paracellular conductance is much greater than the transcellular conductance such that changes in transepithelial conductance are undetectable when a transcellular transport pathway is blocked.

A concentration-response curve of the effects of apical amiloride on $I_{sc}$ shows that inhibitory effects begin at an amiloride concentration of 0.1 $M$ (Fig. 6B). A four-parameter sigmoid curve was fitted to mean values of $I_{sc}$, which drops from 19 $\mu$A/cm$^2$ in the absence of amiloride to 0.3 ± 0.2 $\mu$A/cm$^2$ in the presence of an amiloride concentration of 100 $\mu$M. The amiloride concentration at half-maximal inhibition (IC$_{50}$) was 0.76 $M$. The correlation coefficient of the regression line was 0.9985.

Figure 6C shows the separation of the RT-PCR product of enaC mRNA in agarose gel, revealing a DNA band of 500 bp. After being sequenced, the band turned out to be a DNA
Effects of ouabain on the electrophysiology of monolayers grown in fortified OptiMEM culture medium. In monolayers studied in the presence of fortified OptiMEM containing culture-stimulating agents, the addition of ouabain (1 mM) to the basolateral side inhibited $I_{sc}$ and $V_i$ and decreased $R_t$ (Fig. 7). Unlike the immediate effects of amiloride, the effects of ouabain developed slowly. Under control conditions, $V_i$ was $-9.7 \pm 1.9$ mV, $R_t$ was $1,708 \pm 151 \Omega \cdot \text{cm}^2$, and $I_{sc}$ was $7.2 \pm 0.8 \mu \text{A/cm}^2$ in six DIEC monolayers. After addition of ouabain to the serosal solution (1–2 s), $V_i$ significantly decreased to $-8.2 \pm 1.4$ mV ($P < 0.001$), and $I_{sc}$ significantly decreased to $5.9 \pm 0.8 \mu \text{A/cm}^2$ ($P < 0.001$). Later (20 min), $V_i$ had decreased further to $-0.5 \pm 0.3$ mV ($P < 0.001$, compared with control), $I_{sc}$ had fallen to values not significantly different from zero, and $R_t$ had decreased significantly to $1,106 \pm 111 \Omega \cdot \text{cm}^2$ ($P < 0.01$).

Fig. 6. Effect of apical amiloride on transepithelial electrical variables of DIEC monolayers in fortified OptiMEM. A: linear current ($I$)/voltage ($V$) plots in the absence and presence of amiloride (10 $\mu$M). Amiloride reduces the transepithelial current without affecting the transepithelial conductance. A significant $I_{sc}$ remains at the short-circuit voltage of 0 mV at an amiloride concentration of 10 $\mu$M. Data are means ± SE of 7 monolayers. B: concentration-response curve of the effect of amiloride on $I_{sc}$. The amiloride concentration at half-maximal inhibition ($IC_{50}$) is 0.76 $\mu$M. No significant $I_{sc}$ remains at the amiloride concentration of 100 $\mu$M. $V_i$, voltage clamp; $I_c$, clamp current. C: expression of $\alpha$-epithelial Na$^+$ channel (ENaC) mRNA in DIEC monolayers grown in fortified OptiMEM. The 547-bp cDNA sequence of $\alpha$-ENaC localized at the expected size of the amplified sequence.

Effects of amiloride on transepithelial Na$^+$ fluxes in monolayers grown in fortified OptiMEM culture medium. To examine whether the amiloride-sensitive $I_{sc}$ is carried by Na$^+$, we measured unidirectional, transepithelial isotopic $^{22}$Na$^+$ fluxes in the absence and presence of apical amiloride (Table 1).

Monolayers were bathed on both sides with fortified OptiMEM containing culture-stimulating agents. Under control conditions, the unidirectional Na$^+$ flux from the mucosal to the serosal side ($I_{m\rightarrow s}$) was 18.6 nmol/min$^{-1}$·cm$^{-2}$ and the reverse flux, from serosa to mucosa ($I_{s\rightarrow m}$) was 9.7 nmol/min$^{-1}$·cm$^{-2}$. The net transepithelial Na$^+$ flux was 8.9 nmol/min$^{-1}$·cm$^{-2}$ from mucosa to serosa, which is equivalent to a current of 14.3 $\mu$A/cm$^2$. Because the $I_{sc}$ measured in parallel was only 8.7 ± 1.1 $\mu$A/cm$^2$, the transepithelial Na$^+$ flux significantly exceeds the $I_{sc}$ by a factor of 1.6 ($z = 5.09$, $P < 0.001$).

In the presence of amiloride (10 $\mu$M), the $I_{m\rightarrow s}$ Na$^+$ flux remained near control values, 18.9 ± 2.6 nmol/min$^{-1}$·cm$^{-2}$ ($n = 6$). Likewise, $I_{s\rightarrow m}$ Na$^+$ flux, 11.6 ± 1.7 nmol/min$^{-1}$·cm$^{-2}$, remained near control values ($n = 6$). Accordingly, the net transepithelial Na$^+$ flux was 7.3 nmol/min$^{-1}$·cm$^{-2}$, or 11.7 $\mu$A/cm$^2$, which was not significantly different from control (Table 1). In contrast, amiloride caused $I_{sc}$ to drop significantly to 1.1 ± 0.2 $\mu$A/cm$^2$. Thus amiloride substantially reduced the measured $I_{sc}$ to 13% of control values without affecting the net transepithelial Na$^+$ absorptive flux (Table 1).
Electrophysiological studies of monolayers in plain Ringer solution. When DIEC monolayers in fortified OptiMEM were bathed in Ussing chambers on both sides with plain Ringer solution lacking culture-stimulating agents, the $V_t$ decayed in parallel with a decrease in the $I_{sc}$ (Fig. 8A). Together with four additional experiments, the $V_t$ dropped from $-12.5 \pm 4.6$ to $-1.5 \pm 0.9$ mV ($n = 5$) in 10–30 min. The $I_{sc}$ dropped from $6.6 \pm 1.6$ to $2.0 \pm 0.6$ $\mu$A/cm² ($n = 5$).

Effects of cAMP on monolayer electrophysiology in plain Ringer solutions. Responding to a reviewer, we examined DIEC monolayers for transepithelial Cl⁻ secretion, a functional hallmark of the mammalian jejunum. In these experiments, monolayers were first transferred to plain Ringer solution containing 130 mM Cl⁻ on both sides and amiloride (10 $\mu$M) was added to the mucosal solution (Fig. 8B). Both maneuvers served to abolish the transepithelial Na⁺ current mediated in part by ENaC. Under these conditions, $I_{sc}$ was $0.3 \pm 0.2$ $\mu$A/cm², $V_t$ was $-1.5 \pm 0.1$ mV, and $R_t$ was $3.073 \pm 114$ $\Omega$-cm² in six monolayers (Fig. 8B). Upon the addition of 1 mM DBcAMP to the basolateral solution, $V_t$ and $I_{sc}$ increased gradually and significantly, reaching peak values 5 min later. Within the next 5 min, $I_{sc}$ went to steady-state values of $3.8 \pm 0.2$ $\mu$A/cm², $V_t$ went to $-6.8 \pm 0.4$ mV, and $R_t$ decreased to $1,718 \pm 148$ $\Omega$-cm². The effects of DBcAMP on $V_t$, $R_t$, and $I_{sc}$ are highly significant ($P < 0.001$).

The same experiment repeated in the presence of low-Cl⁻ Ringer solution (13 mM Cl⁻) revealed a marked decrease in the response to DBcAMP (Fig. 8C). In the presence of low-Cl⁻ Ringer on both sides of the monolayer and apical amiloride (10 $\mu$M), the addition of DBcAMP (1 mM) to the serosal side significantly increased $I_{sc}$ from zero to $2.5 \pm 0.1$ $\mu$A/cm² ($P < 0.001$); it increased $V_t$ from $-1.2 \pm 0.1$ to $-3.6 \pm 0.3$ mV ($P < 0.001$), and it significantly decreased $R_t$ from $3,883 \pm 388$ to $1,827 \pm 255$ $\Omega$-cm² ($P < 0.001$) in six monolayers.

Although the effects of DBcAMP were statistically significant, they were less than those observed in the presence of plain Ringer solution with a Cl⁻ concentration of 129.8 mM (Fig. 8, B and C). These results show that the DBcAMP stimulation of $I_{sc}$ and $V_t$ was dependent on the presence of Cl⁻.

The same conclusion was reached in studies of DIEC monolayers in glucose-free Ringer solution and in the presence of apical amiloride (10 $\mu$M). Under these conditions, the addition of 1 mM DBcAMP to the serosal Ringer solution significantly increased $V_t$ from $-1.3 \pm 0.1$ to $-7.1 \pm 0.1$ mV ($P < 0.001$), it significantly increased $I_{sc}$ from $0.1 \pm 0.1$ to $4.6 \pm 0.4$ $\mu$A/cm² ($P < 0.001$), and it significantly reduced $R_t$ from $2,718 \pm 229$ to $1,532 \pm 134$ $\Omega$-cm² ($P < 0.001$), again in six monolayers. Because these effects are quantitatively similar to those observed in the presence of glucose, the significant effects of DBcAMP are not the result of stimulation of electrogenic SGLT.

Expression of SGLT1 in DIEC monolayers. Western blot analysis confirmed the presence of SGLT1 in DIEC cultures (Fig. 9A). In a protein extract of DIEC, the antibody 8821 specific to mammalian SGLT1 recognized a protein band at 75 kDa, where SGLT1 is expected to locate (Fig. 9A, left).
differentiated nature of the IEC culture (40). Furthermore, the cell line lacks most of the active transport systems and the enzymes of the normal epithelium (53).

In the present study, we introduced an intestinal cell culture derived from the normal, healthy intestinal mucosa of the dog jejunum. The DIEC culture was directly produced from jejunal crypts, apparently from committed crypt cells. The culture could be carried through at least six passages without apparent morphological changes or loss of ability to form polarized monolayers on filters. When monolayers are grown on filters, epithelial cells display long and dense microvilli at the apical membrane, extensive basolateral membrane infoldings, and well-defined tight junctions and desmosomes (Figs. 3 and 4).

The expression of keratin no. 18 (Fig. 1c) and the presence of several tight junction proteins confirm the epithelial nature of the culture (Fig. 2). The expression of the epithelial Na⁺ channel ENaC (Figs. 5 and 6) was unexpected and most likely the result of the presence of epidermal growth factor, corticosterone, and insulin in the culture medium (vide infra).

Proteins of the tight junction and the paracellular pathway. To date, 24 members of the claudin protein family have been identified in mice and men (50). The claudins have four transmembrane domains with NH₂ and COOH terminals located in the cytoplasm. Associated with other junctional proteins (occludin, ZO proteins, and junctional adhesion proteins), the claudins define the barrier and permselectivity functions of the paracellular pathway (29, 34). For example, the presence of claudin-16 in tight junctions of the thick ascending limb of the Loop of Henle defines the paracellular Ca²⁺ and Mg²⁺ permselectivity in this part of the nephron (46). More than two claudins may coexist in a tight junction, forming heteropolymers that appear to increase the structural and functional diversity of the paracellular pathway (17).

So far, claudin-3 and claudin-4 have been found in the jejunum (43). Significantly, the same claudins are expressed in the DIEC culture of the dog jejunum, indicating a cultured tight junction that resembles the junction of the normal jejunum. A receptor function has been attributed to these two claudins (5, 26). Binding to the enterotoxin of Clostridium perfringens, claudin-3 and claudin-4 are thought to participate in triggering the diarrhea of the Clostridium infection. Apparently, claudins can have functions in addition to permissive paracellular transport.

Occludin is another tight junction protein with four transmembrane domains. The discrete distribution of occludin in a narrow band surrounding the cells of DIEC monolayers is consistent with its location in tight junctions (Fig. 2b). Likewise, the distribution of ZO-1 is limited to the region of the tight junction (Fig. 2c).

On first examination, the distribution of claudin-4 suggests its presence in the cytoplasm of cell (Fig. 2d). However, upon closer inspection, it appears that the microtome cut takes a tangent to tight junctions. Leaving one epithelial cell, the cut encounters a sharp edge of claudins, suggesting its entry into the tight junction. Passing obliquely through the tight junction, the cut gives rise to the diffuse distribution of claudin-4. In other regions of Fig. 2b, claudin is as discretely confined to the cell border, like occludin and ZO-1, consistent with the expression of claudins at plasma membranes (35). Nevertheless, claudins have been observed inside cells. When exogenous claudin-2 and -4 were experimentally expressed in Madin-
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Darby canine kidney II cells, they were detected in some intracellular, vacuole-like structures in addition to their expression at the cell border, whereas native claudins were restricted to the cell border (9). Studies by Kobayashi et al. (30) suggest that foreign claudins can induce the formation of vacuole-like structures in the cytoplasm.

Desmosomes are “welding spots” that hold cells together and resist tears in epithelial tissues. Desmosomes are abundantly expressed in DIEC monolayers where they outline the lateral interstitial space between epithelial cells and beyond the tight junction (Fig. 2e).

DIEC, a leaky epithelium in spite of appreciable \( R_t \) values. In 1972, Fronter and Diamond introduced the concepts of “leaky” and “tight” epithelia (16). In general, leaky epithelia have 1) low values of \( V_t \) (from 0 to 11 mV) and \( R_t \) (from 6 to 200 \( \Omega \cdot \text{cm}^2 \)), 2) a brush-border apical membrane, and 3) the functional properties of isomotic fluid transport at high rates. In contrast, tight epithelia have high values of \( V_t \) (30 –100 mV) and \( R_t \) (300 –2,000 \( \Omega \cdot \text{cm}^2 \)). Tight epithelia lack a brush border at apical surfaces. The main function of tight epithelia is to generate and maintain high transepithelial concentration differences of solute and water for storage.

In the present study, DIEC monolayers had on average a \( R_t \) of 1,050 \( \Omega \cdot \text{cm}^2 \) consistent with a tight epithelium when compared with 50 \( \Omega \cdot \text{cm}^2 \), the resistance of the small intestine in vivo (15, 36). On the other hand, a low \( V_t \) and the presence of a dense brush border indicate a leaky epithelium. Instead of measures of the \( V_t \) and \( R_t \), Boulapect (6) has proposed a more appropriate criterion of epithelial leakiness or tightness, namely the ratio of transepithelial resistance and paracellular transport in general and the ratio of transepillar and paracellular resistance in particular. To estimate this resistance ratio in DIEC monolayers, we have assumed that the electromotive force of the active transport pathway passing through epithelial cells is 120 mV. Because \( I_{sc} \) is 8.1 \( \mu \text{A/cm}^2 \), it follows that the transepithelial resistance is 14,815 \( \Omega \cdot \text{cm}^2 \). In view of a \( R_t \) (transepithelial and paracellular resistance in parallel) of 1,050 \( \Omega \cdot \text{cm}^2 \), the paracellular resistance is 1,131 \( \Omega \cdot \text{cm}^2 \). Thus the ratio of transepithelial and paracellular resistance indicates a paracellular conductance 13 times greater than the transepithelial conductance consistent with a leaky epithelium. A high paracellular conductance may obscure the effects of amiloride on the \( R_t \) (Figs. 5 and 6).

Functional characterization of DIEC. Absorption of salt, water, and dietary nutrients is the hallmark function of the mammalian small intestine. The human intestine absorbs ~600 mmol Na\(^+\) and 6.5 liters H\(_2\)O/day. The absorption of water is coupled to the absorption of solute. In turn, the absorption of solute relies largely on Na\(^+\)-dependent transport mechanisms such as Na\(^+\)-glucose cotransport, Na\(^+\)-amino acid cotransport, Na\(^+\)/H\(^+\) exchange transport, and NaCl absorption via parallel Na\(^+\)/H\(^+\) and Cl/HCO\(_3\) transport. The transport of Na\(^+\) across the small intestine varies 1) along the length of the intestine (radial heterogeneity), 2) along the length from crypt to the tip of a villus (axial heterogeneity), and 3) between individual epithelial cells in regions of the villus tip and crypt that express transport systems to varying degree (cellular heterogeneity). DIEC monolayers derived from the normal jejunum appear to illustrate some of these heterogeneities. For one reason, the net absorptive isotopic Na\(^+\) flux was nearly two times the \( I_{sc} \) measured in parallel (Table 1), suggesting the presence of electroneutral Na\(^+\) transport mechanisms such as NaCl absorption via parallel Na\(^+\)/H\(^+\) and Cl/HCO\(_3\) transport. For another reason, the inequality of current and flux suggests that flux and current do not derive from the same monolayer area, i.e., the monolayer is heterogeneous like the normal jejunum.

Our preliminary functional characterization of DIEC monolayers illustrated the influence of environmental factors on the expression of transport systems. When DIEC monolayers are grown and studied in OptiMEM solution containing the culture-stimulating agents epidermal growth factor, hydrocortisone, and insulin, the monolayers display an amiloride-sensitive \( I_{sc} \) mediated by ENaC (Fig. 6). This current disappears in plain Ringer solution (Fig. 8A). The subsequent addition of cAMP to the serosal side activates a Cl\(^-\)-dependent current consistent with transepithelial secretion of Cl\(^-\) (Fig. 8B and C). Thus DIEC monolayers can be manipulated to express electrogenic Na\(^+\) absorption under one set of conditions and electrogenic Cl\(^-\) secretion under another.

When grown and studied in fortified OptiMEM solution, the inhibition of the \( I_{sc} \) with amiloride presented from the mucosal side (and not the serosal side, Fig. 5) and the inhibitory effects of serosal ouabain on voltage and \( I_{sc} \) (Fig. 7) outline the rudiments of the Ussing model of epithelial Na\(^+\) transport (51). The amiloride concentration-response curve revealed an \( IC_{50} \) of 0.76 \( \mu \text{M} \) (Fig. 6B), similar to that measured in other epithelia expressing ENaC (4). The immediate effect of ouabain on \( V_t \) and \( I_{sc} \) probably results from the blockade of the electrogenic Na\(^+\)-K\(^+\)-ATPase operating with a stoichiometric exchange of three Na\(^+\) for two K\(^+\). The ouabain-sensitive pump current was measured as 1.3 ± 0.2 \( \mu \text{A/cm}^2 \), which is significantly different from zero (\( P < 0.001 \)). The gradual decline of \( V_t \) and \( I_{sc} \) in the presence of ouabain probably reflects the dissociation of Na\(^+\), K\(^+\), and voltage gradients across cell membranes.

Isotopic Na\(^+\) flux measurements in DIEC monolayers grown and studied in fortified OptiMEM show that ENaC-mediated transepithelial Na\(^+\) transport accounts for only 18% of the net absorptive transepithelial Na\(^+\) transport (Table 1). Because these measurements were made in the absence of \( V_t \) and concentration differences, DIEC monolayers must possess additional transepithelial active transport mechanisms for Na\(^+\). Indeed, an antibody specific to SGLT1 recognizes a protein in DIEC lysates that localizes at the expected position (Fig. 9A). That the same antibody recognizes SGLT1 in the dog jejunum and Cl/HCO\(_3\) transport. For another reason, the presence of ENaC in a cell culture of the small intestine may not be so unusual as it first appears. In rats, the surgical removal of the colon (ileoanal anastomosis) induces
ENaC expression in the ileum (31). Furthermore, the induction of ENaC activity has been attributed to serum- and glucocorticoid-induced kinase that is present in both jejunum and ileum (10).

Next to transepithelial Na\(^+\) absorption, the transepithelial secretion of fluid driven by secretory Cl\(^-\) transport is a functional hallmark of the jejunum. In particular, Cl\(^-\)-driven fluid secretion is thought to take place in the crypt region of villi and is mediated by epithelial cells that harbor Cl\(^-\) channels in the apical membrane and the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter in the basolateral membrane (13, 54). Up to now, at least the following three different Cl\(^-\) channels have been found in the small intestine: the stretch-activated Cl\(^-\) channel, the Ca\(^{2+}\)-activated Cl\(^-\) channel, and cystic fibrosis transmembrane conductance regulator (CFTR) that is activated by phosphorylation via protein kinase A (PKA) and cAMP (3). DIEC monolayers apparently exhibit Cl\(^-\) secretion via CFTR in view of the effects of DBcAMP (Fig. 8, B and C). The nucleotide induced a significant increase in \(I_{sc}\) (from mucosa to serosa) when ENaC channels were blocked with amiloride. The cAMP-stimulated \(I_{sc}\) was dependent on the presence of Cl\(^-\) in the bath medium consistent with the stimulation of transepithelial Cl\(^-\) secretion. Furthermore, the sustained stimulation of \(I_{sc}\) distinguishes CFTR channels from Ca\(^{2+}\)-activated and stretch-activated Cl\(^-\) channels, which respond only transiently to activation (3, 13).

Of note is the significant decrease of the \(R_t\) upon stimulation with cAMP (Fig. 8, B and C). The activation of CFTR in the apical membrane of DIEC monolayers was not expected to decrease the \(R_t\) too much in view of the low paracellular resistance relative to the transcellular resistance that prevented a significant increase in \(R_t\) in the presence of amiloride (Figs. 5A and 6A). Thus cAMP may have affected the paracellular pathway in addition to CFTR in the apical membrane of DIEC. Indeed, the nucleotide is thought to increase tight junction permeability (25). The molecular mechanism is not completely understood, but the PKA phosphorylation of Thr\(^{207}\) of claudin-5, one of the tight junction proteins, is known to trigger the rapid decrease in transendothelial resistance in rat lung endothelium (47).

\(Na^+\)-dependent glucose cotransport via SGLT1 is another hallmark of the jejunum. Glucose enters the cell from the intestinal lumen against its chemical gradient at the expense of its electrochemical gradient across the apical membrane. Glucose leaves the cells across the basolateral membrane down its chemical gradient through another sugar transporter of the family of GLUT glucose transporters (48).

Western blot analysis revealed the presence of SGLT1 in DIEC monolayers. The electrophysiological evidence for SGLT1 was less clear. The addition of 10 mM glucose to DIEC monolayers. The electrophysiological evidence for SGLT1 was less clear. The addition of 10 mM glucose to DIEC monolayers was not expected to increase \(I_{sc}\), but the change was not statistically significant (Fig. 9B). SGLT1 is also present in other cultures of the mammalian intestine, such as Caco-2, HT29 cl.19A, and HT29-D4. However, it is active only in fully differentiated HT29-D4 cell monolayers (12, 18, 20), suggesting that SGLT1 activity correlates with cell differentiation (11). Indeed, SGLT genes are transcribed and translated only in mature enterocytes at villus tips (23). Thus it is likely that the number of tlp cells present in DIEC monolayers may be sufficient to yield a positive Western blot but insufficient to yield significant transepithelial electrical changes when stimulated with glucose.

\(Na^+\)-d-glucose cotransport can be activated by cAMP, as in HRT-18 cells (37). However, in DIEC monolayers, the cAMP stimulation of the \(I_{sc}\) was primarily the result of stimulation of a Cl\(^-\)-dependent current (Figs. 8 and 9).

As a good model for the study of intestinal transport, a monolayer must resemble the in vivo condition physically, morphologically, and biochemically. In the present study, we have examined the morphological and electrophysiological characteristics of a new intestinal culture derived from the normal dog jejunum. DIEC monolayers resemble normal epithelium to a remarkable degree. They display the morphological polarization of the jejunum with a prominent brush border, basolateral membrane infoldings, and the functional polarization of transporters in expected basolateral and apical membrane domains of the jejunum. Likewise, DIEC tight junctions possess the claudin proteins of the normal jejunum. Yet we leave many transport questions open to further studies, and the expression of digestive enzymes has yet to be examined. As a culture of the normal small intestine, DIEC monolayers may find wide application serving basic and applied motivations, from studies of the mechanism and regulation of transepithelial solute and water transport to drug absorption, intestinal clearance of xenobiotics, and high throughput evaluations of pharmaceutical agents.

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