A primary culture of guinea pig gallbladder epithelial cells that is responsive to secretagogues

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Abstract

We have developed a cell culture of guinea pig gallbladder epithelial cells with which to study ion transport. When grown on permeable supports, the cultured epithelia developed a transepithelial resistance (R_t) of ~500 $\Omega\cdot$cm^2. The epithelial cell origin of the cell culture was further confirmed by immunocytochemical localization of cytokeratin. Ionomycin and forskolin increased transepithelial voltage and short-circuit current (I_{sc}) and decreased R_t. The response to ionomycin was transient, whereas that to forskolin was sustained. Both were attenuated by replacement of Cl$^-$ and/or HCO_3^-. Mucosal addition of the anion transport inhibitors DIDS or diphenylamine-2-carboxylic acid (DPC) blocked the response to ionomycin. The response to forskolin was blocked by DPC but not by DIDS. Ionomycin, but not forskolin, increased intracellular Ca^{2+} concentration in fura 2-loaded cells. PGE$_2$, histamine, vasoactive intestinal polypeptide, and secretin elicited a sustained increase in I_{sc}. Responses to ATP and CCK were transient. Thus cultured guinea pig gallbladder epithelia display the range of responses observed in the native tissue and are an appropriate model for studies of ion transport in gallbladder and intestinal epithelia.

Keywords

cultured cells; histamine; ATP; vasoactive intestinal polypeptide; prostaglandin E$_2$; secretin; cholecystokinin; anion secretion; diphenylamine-2-carboxylic acid; 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; ionomycin; intracellular Ca^{2+}; forskolin; cAMP

Cell Culture Systems Provide a homogeneous population of cells that can be grown and studied under defined conditions and manipulated to express foreign proteins. As such, the availability of tissue culture cells has enhanced the ability of investigators to study a wide range of biological and biochemical processes. In gastrointestinal epithelia, cell cultures have been used to study ion transport processes and their regulation by second-messenger pathways. Many of the cell culture systems widely used to study intestinal ion transport, such as T84 (15), Caco-2 (20, 21), and HT-29 (63), are derived from colonic carcinomas.

Although these cells display many of the transport characteristics of the normal colonic tissue type from which they were derived, some investigators have suggested caution in their use because of their tumor origin (17).

Obtaining established cell lines or primary cultures of cells derived from the small intestine that display the transport characteristics of more proximal regions of the intestine has been more problematic. For example, the established non-tumor-derived cell line IEC-6 forms a confluent monolayer but maintains many characteristics of poorly differentiated immature cells (45). The development of transepithelial electric parameters typical of epithelial tissues has not been demonstrated for IEC-6 or the closely related cell line IEC-18 (32, 45).

The gallbladder is an absorptive epithelium that elaborates and stores concentrated bile subsequent to isosmotic absorption of Na^+ and Cl^-. The gallbladder shares many transport characteristics with those observed in the small intestine (5, 49). Both the small intestine and gallbladder are "leaky" epithelia, having a low transepithelial resistance (R_t) and voltage (V_t). Na^+ and Cl^- absorption involves either a neutral Na-Cl carrier and/or dual exchange proteins for Na^+/H^+ and Cl^-/HCO_3^- operating in parallel (11, 13, 19, 27, 36). Agents that elevate intracellular cAMP [e.g., theophylline, prostaglandin, and possibly vasoactive intestinal polypeptide (VIP) and secretin] and in some cases intracellular Ca^{2+} (e.g., ionomycin and ATP) stimulate anion secretion (8, 41–43, 50).

The goal of the present study was to develop a primary culture of guinea pig gallbladder epithelial cells to facilitate studies of the regulation of ion transport by this tissue. Other groups have developed primary cultures of gallbladder epithelia from several species (6, 8, 28, 29, 31, 44), including guinea pig (39). However, to our knowledge there are no previous reports of a systematic study in which these cell cultures have been shown to both develop transepithelial electric parameters and respond to hormones characteristic of the native tissue. The results of this study demonstrate that we have successfully developed a primary culture of guinea pig gallbladder cells that can be used for the systematic investigation of the effects of secretagogues on ion transport in gallbladder epithelia. Given the similarities between the transport processes of leaky epithelia such as gallbladder and small intestine, this cell culture may serve as a useful model with which to study transport processes common to these epithelial cell types.

Materials and Methods

Preparation of guinea pig gallbladder epithelial cell cultures

Excised gallbladders from male Hartley guinea pigs (200–300 g, Harlan Sprague-Dawley, Indianapolis, IN) were stripped of underlying muscle and minced. The tissue was incubated in an isolation solution containing 0.005% pronase and 0.1% BSA at 37°C, and the solution was triturated at intervals to facilitate cell isolation. After 1 h, the isolated cells were decanted and washed in three changes of sterile DMEM-Ham's F-12 media containing 20% FBS (Life Technologies, Rockville, MD), ITS media supplement (5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenium), 0.05 mg/ml hydrocortisone, 2 mM glutamine (Fisher Scientific, Atlanta, GA), and 0.1% penicillin-streptomycin (Life Technologies) (47). Cell viability following isolation as assessed by trypan blue exclusion was >95%. Before seeding

on glass coverslips or permeable Transwell supports (Costar, Cambridge, MA), the isolated cells were briefly placed in plastic culture flasks to decrease fibroblast contamination. Transwells (0.78 cm^2; Costar 3407) were coated with the basement membrane extract Matrigel (Becton Dickinson Labware, Bedford, MA) before seeding. Cells isolated from the gallbladders of two guinea pigs were pooled and yielded a sufficient number of cells to seed 18 Transwells at a density of 3.0×10^5 cells/Transwell in 200 μl of supplemented DMEM-Ham's F-12 medium.

Immunocytochemistry

Cells grown on glass coverslips were fixed and permeabilized with ice-cold methanol. Cells were first incubated with a primary antibody that recognizes cytokeratin (mouse anti-keratin monoclonal AE1/AE3; Boehringer Mannheim, Indianapolis, IN). The secondary antibody (anti-mouse IgG, F_{ab}-specific TRITC conjugate) was then applied. Cells to which only the secondary antibody was applied without prior addition of primary antibody served as controls. Fluorescence localization of cytokeratin was observed using a Molecular Dynamics Multiprobe 2001 inverted confocal laser microscope with the appropriate setting for Cy3 fluorescence. Laser intensity was adjusted to the lowest attenuation that permitted a strong specific signal and minimal background without significant photobleaching of the dye. The images were digitized, stored, and analyzed using a Silicon Graphics Iris Indigo workstation and printed using a Mitsubishi color video copy processor.

Transepithelial electric parameters of cultured monolayers

Gallbladder epithelial cell cultures grown on Transwells were mounted into Lucite Ussing chambers (Costar) and bathed by a physiological Ringer solution. The V_t and short-circuit current (I_{sc}) were monitored by means of an automatic voltage clamp device (Model VCC-600; Physiologic Instruments, San Diego, CA) in contact with the bathing solutions via Ag-AgCl electrodes inserted into KCl- or Ringer-agar bridges. Experiments were conducted under open-circuit [transepithelial current (I_t) = 0] or short-circuit conditions (V_t = 0) except for brief periods during which V_t or I_t was clamped to ±5 mV or 5 μA, respectively. Under both conditions, reported values of V_t and I_{sc} are measured rather than calculated values. R_t was calculated from deflections in V_t produced by bipolar V_t/I_t pulses using the relation $R_t = V_t/ I_t$. Secretagogues were added directly to both half-chambers from stock solutions to give appropriate final concentrations. Anion transport inhibitors were added to the apical/mucosal bathing solution. When secretagogues or inhibitors were dissolved in DMSO or ethanol, an equal volume of solvent was added to control cultures. Cultures were equilibrated in the chamber for 20–30 min before the addition of secretagogues or inhibitors. Normal Ringer solution contained (in mM) 140 Na$^+$, 5 K$^+$, 25 HCO_3^-, 1 Ca^{2+}, 1 Mg^{2+}, and 124 Cl$^-$. HCO_3^--free solutions contained (in mM) 140 Na$^+$, 5 K$^+$, 1 Ca^{2+}, 1 Mg^{2+}, 124 Cl$^-$, and 10 HEPES. For low-Cl$^-$ solutions, gluconate replaced Cl$^-$ to a final Cl$^-$ concentration of 4 mM. HCO_3^--containing solutions were gassed with 95% O$_2$-5% CO$_2$. HCO_3^--free solutions were gassed with 100% O$_2$. Experiments were conducted at 37°C (pH = 7.4).

Measurement of intracellular Ca^{2+}

Cultured gallbladder cells plated on coverslips were incubated with fura 2-AM (Molecular Probes, Eugene, OR) for 30 min at 37°C. In some cases, the loading solution contained 5 mM Probenecid to enhance dye uptake. The cells were washed once with HEPES-based Ringer and placed on the stage of a digital-imaging microscope. The dual-wavelength ratiometric technique (23, 55) was used to measure the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Cellular images were acquired on a Zeiss IM-35 microscope (Carl Zeiss Instruments, Norcross, GA) using a Nikon 40× objective (Southern Micro Instruments, Atlanta, GA) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The images were collected with a Dage ISIT 66 video camera (Dage, Michigan City, IN) and analyzed using Inovison IC300 software (Inovision, Research Triangle, NC) running on a SPARCstation2 workstation (Sun Microsystems, Mountain View, CA). Digitized images were corrected for background fluorescence. The dye within the guinea pig gallbladder cells appeared to be fully Ca^{2+} sensitive. Thus $[Ca^{2+}]_i$ was calculated by extrapolation from a standard curve that had been generated from the measurement of the fluorescent ratio of aliquots of fura 2-free acid over a range of Ca^{2+} concentrations. Fluorescent ratios of the aliquots were acquired at the same gain setting on the camera that was used for the acquisition of cellular fluorescent ratios.

Reagents

All were purchased from Sigma-Aldrich Chemical (St. Louis, MO) unless otherwise specified.

Statistics

Results are given as means ± SE. The statistical significance of differences was determined from paired or unpaired Student's t-test or ANOVA as appropriate. Values of $P < 0.05$ were considered statistically significant. In all cases, n represents the number of experiments.

Results

When cultured under appropriate conditions, isolated guinea pig gallbladder epithelial cells developed a confluent monolayer. Contamination by nonepithelial cells was minimized by mechanically stripping the mucosal layer of underlying musculature and preplating cells on plastic to remove fibroblasts. Cell proliferation required the presence of serum in the culture media because cells cultured in a similar defined serum-free medium supplemented with epidermal growth factor remained viable for several days but failed to proliferate. With the inclusion of 20% FBS in the culture medium cell, nidi (5–10 cells) appeared in 1 day that subsequently became confluent in 4–5 days.

The epithelial origin of the resulting cell culture was confirmed from the localization of the epithelial cell marker cytokeratin in the cultured cells by immunocytochemistry using a fluorescently labeled antibody and confocal microscopy. Figure 1 shows the filamentous pattern of intracellular cytokeratin typical of epithelial cells. No fluorescence was observed in control cells, to which only the secondary antibody was applied (data not shown).

To evaluate functional similarities between cultured epithelia and the native tissue, cells were grown on Transwells for assessment of transepithelial electric characteristics. R_t increased with time in culture, reaching a maximal resistance of 650 ± 99 Ω in 4 days, corresponding to the time of confluence (Fig. 2). Given a Transwell surface area of 0.78 cm^2, this corresponds to a R_t of ~ 500 $\Omega \cdot cm^2$, a value higher than that reported for the native tissue (24, 27). R_t remained stable up to *day 14* and then declined. The time course of changes in R_t was also used to evaluate the effect of serum concentration on proliferation and confluence. Although there was no effect of serum concentration in the range of 5–20% FBS on the time at which the cultures reached the maximal R_t, R_t was significantly higher at *day 14* for cells grown in 20 vs. 5% FBS ($P < 0.05$).

Cultures grown on Transwells having a $R_t > 300$ Ω were placed in modified Ussing chambers, and the responses to either 1×10^{-6} M ionomycin or 5×10^{-5} M forskolin (which elevate intracellular Ca^{2+} and cAMP, respectively) were evaluated. Figure 3 and Table 1 show that ionomycin produced transient changes in V_t and in I_{sc}, respectively, whereas forskolin produced a biphasic, sustained increase. In both cases, R_t tended to decrease at the peak of the changes in I_{sc} and then returned toward normal (Fig. 4). These changes are similar to the changes observed in native tissue that result from a stimulation of anion secretion (26, 42, 51).

Ion substitution experiments were conducted to determine the ionic dependency of the ionomycin- and forskolin-stimulated I_{sc}. Figure 5 shows that replacing both Cl^- and HCO_3^- significantly inhibited ionomycin and forskolin responses. The forskolin-stimulated I_{sc} was significantly attenuated when either Cl^- or HCO_3^- was replaced. These results are expected if anion secretion underlies the changes in I_{sc}.

To further evaluate the contribution of anion secretion to the ionomycin- and forskolin-stimulated I_{sc}, we determined the effect of anion transport inhibitors (Fig. 6). Anion transport inhibitors were added to the mucosal bathing solution. The response to ionomycin was blocked by DIDS (1×10^{-4} M) and diphenylamine-2-carboxylic acid (DPC; 5×10^{-5} M), whereas the response to forskolin was blocked by DPC but not DIDS. This sensitivity to anion transport inhibitors provides additional evidence that the ionomycin- and forskolin-stimulated I_{sc} represents anion secretion.

Changes in I_{sc} following the addition of ionomycin and forskolin should reflect independent activation of Ca^{2+}- and cAMP-dependent pathways, respectively. To confirm this, we evaluated the effect of ionomycin and forskolin on $[Ca^{2+}]_i$ in cells loaded with the fluorescent Ca^{2+} indicator fura 2 with the use of fluorescent video microscopy (Fig. 7). Resting $[Ca^{2+}]_i$ was 60 nM. Ionomycin caused a sustained increased $[Ca^{2+}]_i$ to 1.3 μM, whereas forskolin had no effect.

Ionomycin and forskolin modulate ion transport by affecting intracellular Ca^{2+} and cAMP levels, respectively, at intracellular sites downstream of the binding of a secretagogue or transport modulator to its plasma membrane receptor. To evaluate the plasma membrane receptor profile of cultured gallbladder epithelia, we assessed the effect of several modulators of gastrointestinal ion transport on V_t and I_{sc}. Figure 8 shows typical changes in

V_t in response to the addition (indicated by the arrows) of PGE$_2$ (10^{-6} M), histamine (10^{-4} M), VIP (10^{-8} M), secretin (10^{-7} M), and ATP (10^{-4} M). The effect of these secretagogues on mean V_t and I_{sc} is shown in Table 1. Responses to PGE$_2$, histamine, VIP, and secretin were sustained as was the case for forskolin. ATP and CCK elicited transient responses similar to that observed for ionomycin. Carbachol (10^{-3} M) and glucagon (10^{-6} M) had no effect on the transepithelial electric parameters (data not shown).

Discussion

The goal of this study was to develop a primary culture of guinea pig gallbladder epithelial cells with which to study the regulation of ion transport in mammalian gallbladder. Our criteria for achieving this goal included demonstrating that the cell culture *1*) develops a confluent monolayer of epithelial cells that is stable over time, *2*) develops a R_t, V_t, and I_{sc}, *3*) lends itself to the application of several different experimental techniques, and, importantly, *4*) displays the range of responses associated with the native tissue. The data support our success in developing a primary cell culture that meets these criteria.

First, immunocytochemistry demonstrated an intracellular distribution of cytokeratin in cultured gallbladder cells typical of epithelial cells. The localization of cytokeratin is widely used as a marker for epithelial cell origin (18, 39, 40). The cell culture reaches confluency in 4 days and is stable for 5–7 days. Second, cell cultures grown on permeable supports develop transepithelial electric parameters V_t, R_t, and I_{sc}. Thus the effects of various transport modulators on ion transport can be assessed from changes in these parameters. Third, we have demonstrated that the primary culture can be used in a number of techniques required for studies of the regulation of ion transport. This includes the Ussing I_{sc} technique and the use of fluorescence indicator dyes that offer a convenient measure of intracellular ion concentrations such as Ca^{2+}. Although not shown in this study, we have also successfully initiated patch clamp studies in both whole cell and cell-attached configurations to further characterize cells derived from cultured monolayers.

Fourth, and importantly, the primary cultures of guinea pig gallbladder epithelial cells display responses to transport modulators observed for the native tissue. For example, agents that elevate intracellular cAMP (e.g., theophylline, forskolin, prostaglandin, and possibly VIP and secretin) and in some cases Ca^{2+} (e.g., ionomycin and ATP) stimulate anion secretion by *Necturus*, guinea pig, porcine, and human gallbladder (8, 28, 41–43). Mechanisms underlying secretion are typical of secretory epithelia in that Ca^{2+} and cAMP open anion-conductive pathways in the apical membrane (28, 42, 43, 50, 51). Our results clearly demonstrate appropriate responses of cultured monolayers to agents that elevate Ca^{2+} and cAMP, ionomycin, and forskolin, respectively. These agents increased V_t and I_{sc} and reduced R_t. The ionomycin- and forskolin-stimulated I_{sc} were attenuated by replacement of bath Cl$^-$ and/or HCO$_3^-$ and blocked by anion transport inhibitors, consistent with stimulation of anion secretion. The ionomycin- and forskolin-stimulated responses differed in anion transport inhibitor sensitivity, ability to elevate [Ca^{2+}]$_i$, and time course, suggesting activation of distinct pathways. In the absence of measurements of ion fluxes, we cannot exclude alternative interpretations for the results of anion substitution and blocker experiments (e.g., effects on cation fluxes subsequent to changes in intracellular pH).

However, activation of anion secretion is the most plausible explanation consistent with our data and the literature (51).

The different sensitivity of ionomycin- and forskolin-stimulated responses to the anion transport inhibitors DPC and DIDS observed in this study suggests similarities between Cl^- efflux pathways in human and guinea pig gallbladder epithelia. In our studies, DPC and DIDS blocked the ionomycin-stimulated increase in I_{sc}. Although the forskolin-stimulated response was blocked by DPC, DIDS was ineffective. Inhibition by DPC of cAMP-stimulated HCO_3^- secretion in the native gallbladder epithelia has been attributed to an effect on apical Cl^-/HCO_3^- exchange (42). In human gallbladder epithelia (8), differential sensitivity of Ca^{2+}- and cAMP-dependent Cl^- secretion to anion transport inhibitors was suggested to reflect activation of separate Cl^- efflux pathways. This appears to be the case in a number of secretory epithelia, in which DIDS has been observed to block Ca^{2+}-dependent Cl^- currents and channels (1, 2) but has no effect on cAMP-dependent Cl^- currents and channels (1, 2, 4, 7, 9, 14, 16). On the other hand, DPC blocks both Ca^{2+}- and cAMP-dependent Cl^- currents and channels (1, 4, 7). The molecular species underlying DIDS-sensitive currents are unclear; however, DPC-sensitive, cAMP-dependent currents appear to be associated with the cystic fibrosis transmembrane conductance regulator CFTR (4, 12, 48). In *Necturus* gallbladder, anion transport inhibitors (28, 30, 53) did not block cAMP-dependent Cl^- currents. Thus cultured guinea pig gallbladder epithelial cells closely resemble cultured human gallbladder epithelial cells (8).

Given that anion secretion appears to underlie both ionomycin- and forskolin-stimulated I_{sc}, the observed differences in the time course of changes in I_{sc} would not be expected. Forskolin elicited a biphasic, sustained increase in V_t and I_{sc} and a fall in R_t. Ionomycin elicited similar but transient changes in transepithelial electric parameters. In bovine pancreatic duct cells and T84 cells, agents that increase $[Ca^{2+}]_i$ elicit a transient and rapid increase in I_{sc}, whereas the response to agents that elevate cAMP is delayed but sustained (1, 14, 16). The mechanisms underlying these differences are unclear. One possible explanation is that K^+ efflux occurring concomitantly with anion secretion blunts the change in I_{sc} related to anion secretion. In support of this, ionomycin-stimulated K^+ efflux is five times that elicited by PGE_2 in native guinea pig gallbladder (26). An alternative explanation for the transient nature of the I_{sc} response to ionomycin is that some inhibitory pathway activated by Ca turns off secretion, as suggested by Barrett (3) and Wang et al. (60) for T84 cells.

To evaluate the plasma membrane receptor profile of cultured gallbladder epithelia, we assessed the effect of several modulators of gallbladder ion transport on V_t and I_{sc}. PGE_2, histamine, VIP, secretin, and ATP stimulated I_{sc} at concentrations observed to be effective on native guinea pig gallbladder epithelia and other anion secreting epithelia (33–35, 41, 43, 46, 51, 52, 58). CCK also stimulated the I_{sc}, but carbachol and glucagon had no effect on the transepithelial electric parameters. To our knowledge, carbachol and glucagon have not been reported to stimulate I_{sc} in native guinea pig gallbladder. ATP and CCK elicited a transient response similar to that observed for ionomycin. Responses to PGE_2, histamine, VIP, and secretin were sustained, as was that for forskolin. These similarities suggest that PGE_2, histamine, VIP, and secretin activate cAMP-dependent pathways, whereas ATP and CCK

activate Ca^{2+}-dependent pathways in cultured guinea pig gallbladder epithelial cells. Elevation of cAMP by PGE_2, VIP, and secretin has been observed for gallbladder epithelial cells from several species (41, 43). Likewise, elevation of $[Ca^{2+}]_i$ by ATP was observed for several anion secretory epithelia in which ATP acts at plasma membrane purinergic receptors (8, 59, 64). In *Necturus* gallbladder, ATP increased apical and basolateral K^+ conductance and increased apical Cl^- permeability (10). Chinet and colleagues (8) recently demonstrated the presence of P2Y2 purinoceptors on cultured human gallbladder cells, the activation of which elicits anion secretion through activation of Ca^{2+}-dependent pathways. In preliminary studies, we have observed increased $[Ca^{2+}]_i$ following ATP addition to fura 2-loaded cells (25).

The response of cultured gallbladder epithelial cells to histamine and CCK also resembles that of forskolin and ionomycin, respectively. However, the second-messenger pathways activated by histamine and CCK in gallbladder epithelial cells are less clear. In some epithelial cell types, histamine elicits anion secretion by binding to H_1 receptors and activating Ca^{2+}-dependent signaling pathways (46, 52), whereas in others both Ca^{2+}- and cAMP-dependent pathways are activated (33, 54, 61). The increase in cAMP was shown to result from eicosanoid production, which then stimulated ion transport. Although the effect of CCK on anion secretion in gallbladder epithelial cells has not been reported, in pancreatic acinar cells CCK stimulates secretion via activation a Ca^{2+}-dependent pathway (62). Together, these results demonstrate a physiological role for these agents in regulating cultured gallbladder epithelial ion transport and, importantly, that the cell culture is an appropriate model system for the native tissue.

Although the responses of cultured cells to secretagogues were similar to those of the native tissue, the cultured guinea pig gallbladder epithelia differed from the native tissue with respect to R_t. R_t was considerably higher in cultured epithelia, at 500 vs. 50 $\Omega \cdot cm^2$ (24, 51). Presently, we do not have an explanation for this observation. However, a comparison of the resistance of cultures grown on filters to resistance of native tissue is complicated by the elaboration of the surface area of native tissues by the presence of folds and ridges. This would lead to an underestimate of true surface area and, therefore, resistance in native tissue. We also cannot discount the possibility that our culture conditions (e.g., matrix, growth factors, etc.) select for a specific cell type or change some of the monolayer characteristics. Alternatively, the difference in R_t may reflect the absence of a factor that normally regulates junctional permeability. It is important to note, however, that the response of the R_t to secretagogues is identical to that of native gallbladder epithelia.

To our knowledge, this is the first systematic study in which a gallbladder epithelial cell culture has been shown to develop transepithelial electric parameters and to respond to hormones characteristic of the native tissue. Other groups have developed primary cultures of gallbladder epithelia from several species for use in a variety of different studies (6, 8, 28, 29, 31, 39, 44). Most pertinent to the present study, O'Brien et al. (39) demonstrated that epithelioid outgrowths from guinea pig gallbladder explants expressing cytokeratin could be maintained for 10–14 days in culture. These cells failed to form confluent monolayers and were not characterized beyond preliminary single-channel measurements. Details

concerning the culture conditions were not given; thus we cannot speculate as to differences in our culture conditions.

Many cell culture systems presently used to study regulation of intestinal epithelial ion transport are of colonic tumor origin (17). In addition, small intestinal cell lines derived from fetal tissue such as IEC-6 and IEC-18 cells display immature phenotypes (32, 45). Gallbladder and small intestinal epithelial cells have a number of similarities with respect to ion transport mechanisms and regulation. Like guinea pig gallbladder, the mammalian small intestine absorbs Na^+ by an electroneutral process, secretes HCO_3^-, and responds similarly to intestinal hormones and transport modulators such as VIP, secretin, histamine, and PGE_2 (reviewed in Refs. 5 and 49). Given this, the primary gallbladder epithelial cell culture described in this study may provide a new model with which to examine mechanisms and regulation of ion transport common to these epithelia.

In summary, our laboratory has developed a primary culture of guinea pig gallbladder cell that displays transport processes and hormonal responses typical of the native tissue. The cultured guinea pig gallbladder cells can be studied using the wide range of techniques currently available to study epithelial ion transport and its regulation. As such, the preparation provides a useful model system with which to study transport in gallbladder and intestinal epithelial cells.

Acknowledgments

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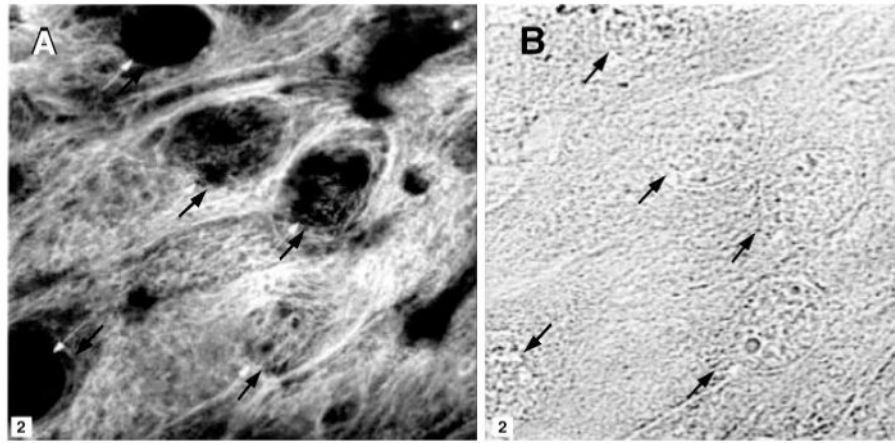
Fig. 1.
Localization of cytokeratin in cultured guinea pig gallbladder cells. *A*: fluorescence image. *B:* corresponding bright-field image. The box marked on each image is 2 μm^2. The arrows mark the locations of individual nuclei for the alignment of the 2 images.
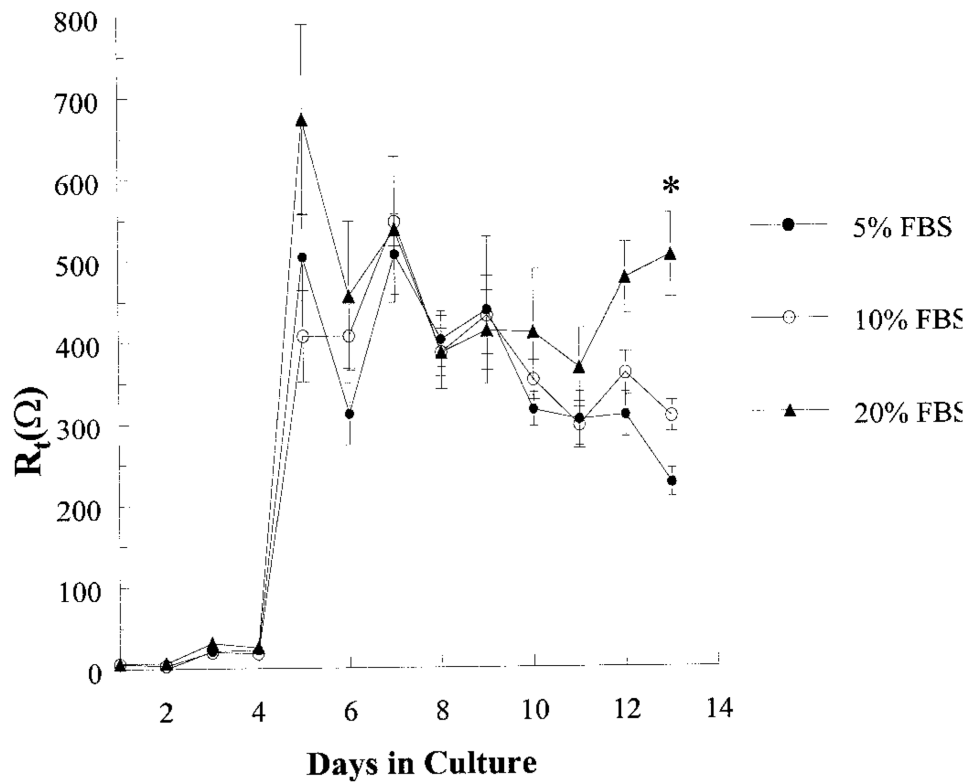
Fig. 2.
Development of transepithelial resistance (R_t) for guinea pig gallbladder epithelial cells grown in 5, 10, or 20% FBS with respect to days in culture. Cells from the same preparation were cultured in triplicate either in 5, 10, or 20% FBS, and the R_t was measured daily for 14 days. Each point represents the mean ± SE of 4 experiments (separate preparations). *Significant difference between 5 and 20% FBS.
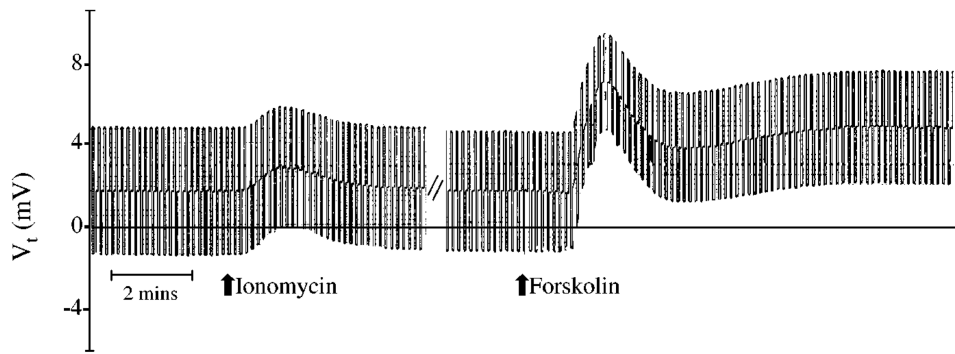
Fig. 3.
Response of cultured guinea pig gallbladder epithelial monolayers to 1×10^{-6} M ionomycin and 5×10^{-5} M forskolin. The deflections in transepithelial voltage (V_t) result from bipolar current pulses used to measure R_t. The steady-state V_t is represented by the midpoint of the deflections. Drugs were added as indicated at the arrows. Results are shown for a typical experiment.
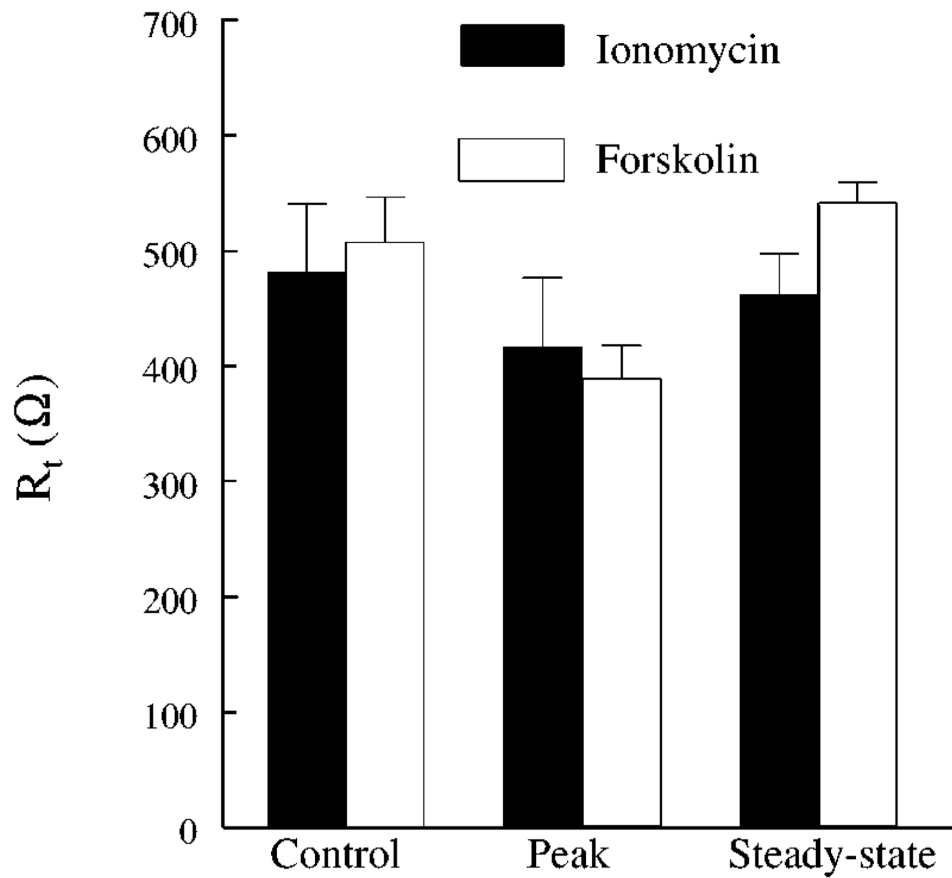
Fig. 4.
R_t before (control) and after (peak and steady-state) the addition of 1×10^{-6} M ionomycin and 5×10^{-5} M forskolin. Each bar represents the mean ± SE of 7 Transwells from 3 different preparations.
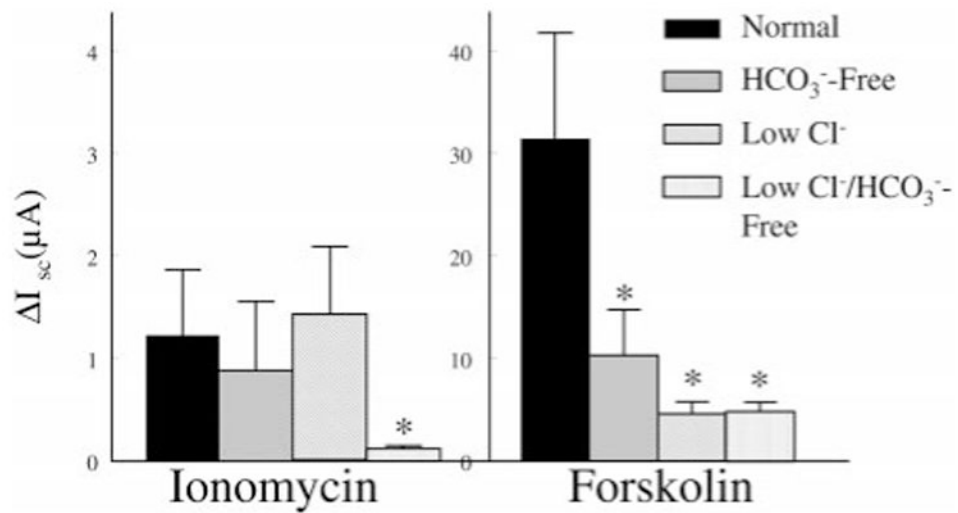
Fig. 5.
Effect of ion substitution on peak changes in short-circuit current (I_{sc}) following 1×10^{-6} M ionomycin or 5×10^{-5} M forskolin. Each bar is the mean ± SE of 7 Transwells from 3 different preparations. * Significant difference from normal Ringer solution.
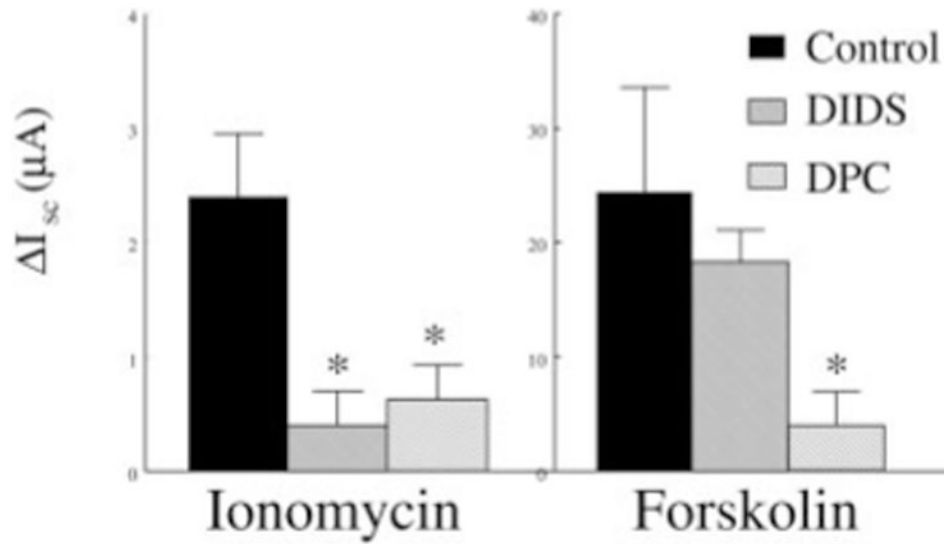
Fig. 6.
Effect of anion transport inhibitors on ionomycin- and forskolin-stimulated I_{sc}. Blocker concentrations were 1×10^{-5} M for DIDS and 5×10^{-5} M for diphenylamine-2-carboxylic acid (DPC). Each bar is the mean ± SE of 7 Transwells from 3 different preparations. *Significant difference from controls.
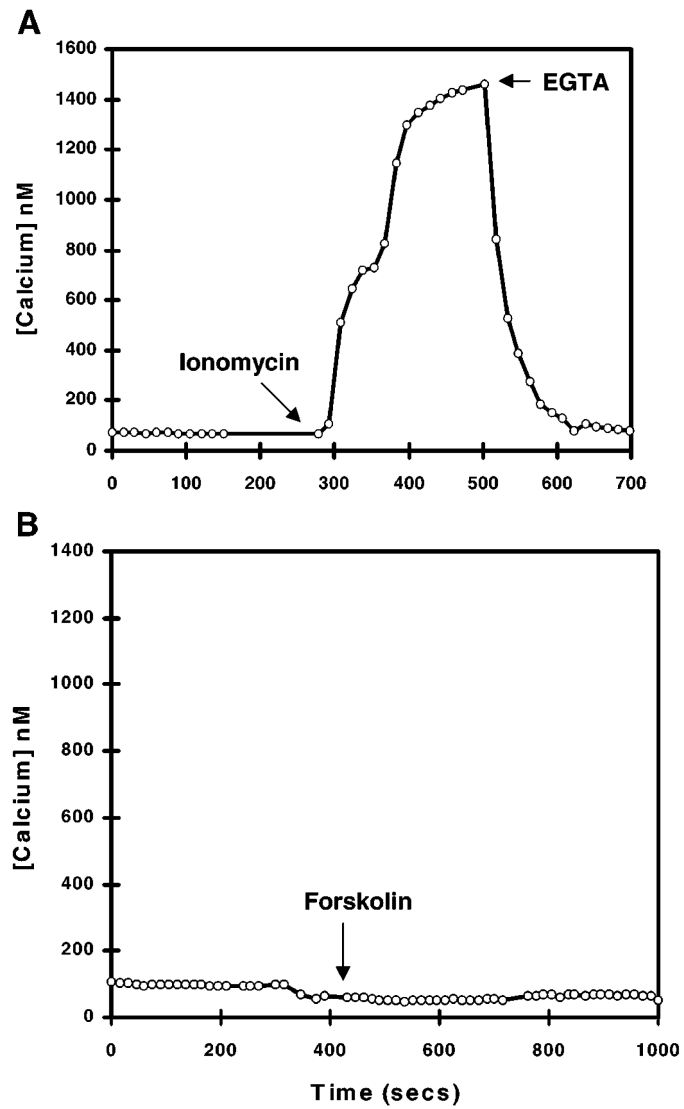
Fig. 7.
Changes in intracellular Ca^{2+} concentration ([Calcium]) in fura 2-loaded cultured guinea pig gallbladder epithelial cells following the addition of 1×10^{-6} M ionomycin (*A*) or 5×10^{-5} M forskolin (*B*). Ionomycin and the Ca^{2+} chelator EGTA were added after forskolin to demonstrate that the cells and measuring system were functioning appropriately (data not shown).
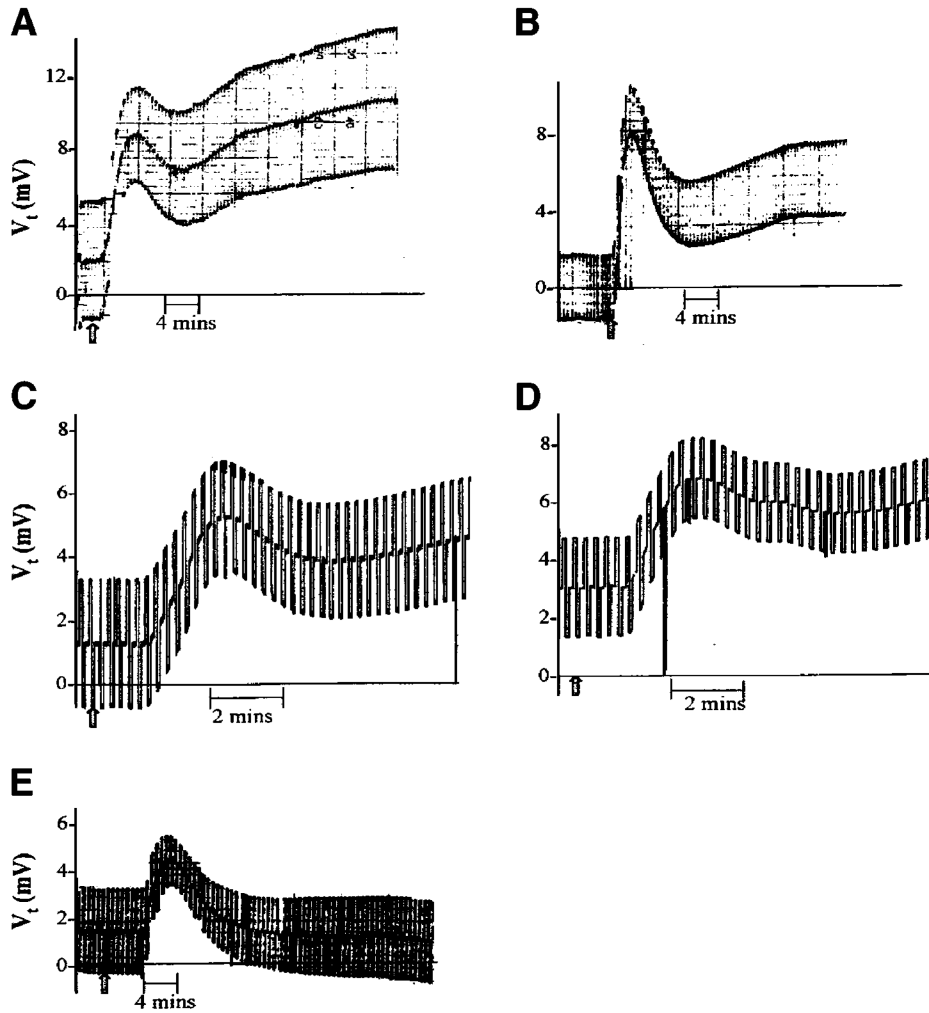
Fig. 8.
Change in V_t in response to 1×10^{-6} M PGE_2 (*A*), 1×10^{-4} M histamine (*B*), 1×10^{-8} M vasoactive intestinal polypeptide (*C*), 1×10^{-6} M secretin (*D*), and 1×10^{-4} M ATP (*E*). The deflections in V_t result from bipolar current pulses used to measure R_t. The steady-state V_t is represented by the midpoint of the deflections. Drugs were added as indicated at the arrows. Data are for typical experiments. Changes in I_{sc} are qualitatively similar to changes in V_t. The mean I_{sc} and V_t data are shown in Table 1.

Table 1

Effect of gastrointestinal secretagogues on V_t and I_{sc} of cultured guinea pig gallbladder epithelial cells

	Basal		Peak		Steady State		
	V_t	I_{sc}	V_t	I_{sc}	V_t	I_{sc}	n
Forskolin	1.4 ± 0.5	3.2 ± 1.4	$8.4 \pm 1.1^*$	$28.1 \pm 3.8^*$	$6.7 \pm 1.4^*$	$18.4 \pm 3.6^*$	6
PGE_2	2.0 ± 0.9	6.5 ± 2.1	6.0 ± 2.2	$16.9 \pm 2.2^*$	7.5 ± 2.6	$16.5 \pm 1.7^*$	5
Histamine	1.8 ± 0.2	6.4 ± 1.7	$4.8 \pm 0.7^*$	$20.1 \pm 4.3^*$	$3.8 \pm 0.4^*$	$15.7 \pm 3.4^*$	7
VIP	3.7 ± 0.3	12.0 ± 2.4	7.3 ± 2.5	$21.2 \pm 3.0^*$	7.8 ± 3.0	$21.5 \pm 3.8^*$	4
Secretin	3.6 ± 1.1	5.6 ± 1.9	$7.7 \pm 1.4^*$	$28.2 \pm 3.4^*$	6.9 ± 2.0	16.3 ± 3.3	5
Ionomycin	2.1 ± 0.7	5.8 ± 0.4	2.8 ± 0.6	$8.4 \pm 0.6^*$	1.8 ± 0.5	6.3 ± 0.6	6
ATP	2.8 ± 0.3	4.2 ± 0.3	$4.9 \pm 0.5^*$	$9.8 \pm 0.7^*$	3.5 ± 0.6	5.4 ± 0.6	4
CCK	1.2 ± 0.1	6.1 ± 1.4	$1.9 \pm 0.2^*$	$8.5 \pm 1.3^*$	$1.4 \pm 0.2^*$	6.7 ± 1.4	3

Values are means $\pm$ SE; n = no. of experiments. Secretagogues were added to both half-chambers. Transepithelial voltage (V_t) and short circuit current (I_{SC}) are measured as described in MATERIALS AND METHODS. Ionomycin, ATP, and CCK elicited transient increases in V_t and I_{SC}. Responses to forskolin, PGE_2, histamine, vasoactive intestinal polypeptide (VIP), and secretin were sustained.

* $P < 0.05$ vs. basal V_t or I_{SC}. In each secretin experiment, the steady-state I_{SC} exceeded the basal I_{SC}, although the difference was not statistically significant.