A human colonic tumor cell line that maintains vectorial electrolyte transport

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A human colonic epithelial cell line, Tg4, derived from a colonic carcinoma, has been examined both morphologically and functionally. The cells grew to confluence as a monolayer with the basolateral membrane attached to the surface of the culture dish and a microvillus-studded apical membrane facing the media. Tight junctions and desmosomes were demonstrated between adjacent cells. Confluent monolayer cultures conducted vectorial electrolyte transport that could be altered by a variety of secretagogues and antisecretagogues similar to isolated intestinal tissues. This cell line will serve as an excellent model system for the study of electrolyte transport processes and their regulation by peptide hormones and neurotransmitters.

colon; cell culture; gastrointestinal hormones; neurotransmitters

STUDIES OF TRANSPORT PHENOMENA and the intracellular processes involved in their regulation will be facilitated greatly by the availability of a continuous supply of uniform epithelial cells. The uniformity of a cell line in culture eliminates problems created by mixed cell types and different degrees of viability, which cannot be eliminated when whole isolated intestinal tissue or isolated intestinal cells are studied. Current cell-culture techniques make it possible for our laboratory to culture and maintain a few human colonic epithelial cell lines (12, 16). In this report, we examine one of these cell lines, Tg4, both morphologically and functionally. Morphologically, we have demonstrated its similarity to intact or isolated intestinal tissues. Functionally, the cells responded to a variety of peptide hormones or neurotransmitters, indicating the presence of receptors and intact transport processes for electrolytes.

METHODS

Cell culture. The Tg4 cell line is a transplantable human colonic carcinoma cell line that was established in BALB/c nude mice. Originally, the tumor specimens were obtained from lung metastases in a patient with colonic carcinoma and injected subcutaneously into nude mice. Light microscopic characteristics and hormonal requirements for growth have been described previously (12, 16).

For the studies reported here, Tg4 cells were grown as monolayers in a 1:1 mixture of Dulbecco-Vogt modified Eagle's medium and Ham's F-12 medium supplemented with 15 mM HEPES buffer (pH 7.5), 1.2 g NaHCO3, 40 mg penicillin, 8 mg ampicillin, and 90 mg streptomycin/l and 6% newborn calf serum. The media did not contain amphotericin. Confluent monolayers were subcultured every 7–14 days by trypsin treatment with 0.1% trypsin and 0.9 mM EDTA in Ca2+- and Mg2+-free, phosphate-buffered saline.

For the Ussing chamber studies described below, cells were plated after trypsin treatment on rat tail collagen-coated Nitex mesh attached to a Lexan ring.

Electron microscopic studies. For the transmission electron microscopic study, Tg4 cells were grown to confluence in 35-mm culture dishes. For scanning electron microscopy, cells were grown on glass cover slips in culture dishes. Cells were fixed in situ with modified Karnovsky's fixative [2% paraformaldehyde and 1.5% glutaldehyde in 0.1 M cacodylate buffer (pH 7.2)] at 4°C for 1 h and then postfixed with 1% OsO4 in the same buffer at room temperature for 2 h. Cells were then dehydrated through graded ethanol. For transmission electron microscopy, the fixed, dehydrated cells were embedded in Epon 812, and thin sections were cut perpendicularly to the substrate and stained with uranyl acetate and lead citrate. Micrographs were taken with a Hitachi HU12A electron microscope at 75-kV acceleration voltage.

For freeze-fraction electron microscopy, Tg4 cells were grown to near confluence on 60-mm plates, washed free of growth medium with two changes of phosphate-buffered saline, and treated with trypsin. After detachment,
cells were collected by centrifugation (250 g for 5 min). The trypsin solution was then aspirated and the cell pellet was washed twice with phosphate-buffered saline by centrifugation. The cell pellet was then fixed in phosphate buffered saline containing 1% glutaraldehyde for 2.5 h at 4°C. After fixation, the cell pellet was washed three times with phosphate-buffered saline, and then phosphate-buffered saline containing 10% glycerol was added as a cryoprotective agent. After 30 min at 4°C, the glycerol concentration was raised to 20% for an additional 30 min. Then, the level of glycerol was elevated to 30%, and cells were left at this concentration at 4°C for 60 min. Samples were quick-frozen on Balzer’s gold-welled specimen supports in Freon 22 maintained near its freezing point by liquid nitrogen. Fracturing was done in a Balzer’s 300 Freeze-Etch, equipped with a platinum evaporating electron gun, as described by Wiley and Ellisman (19). A 2 nm thick platinum layer was deposited on the samples, followed by an additional stabilizing 40-nm-thick carbon layer. After bleaching, the replicas were examined at 100 kV with a Joel 100 CX electron microscope.

Ussing chamber. The Ussing chamber was modified so that it would be suitable for handling cell monolayers. A diagram of the modified chamber is shown in Fig. 1. Modification of the Ussing chamber was necessary to maintain the integrity of the cell monolayers during the study. It was designed to minimize the water turbulence created by the air-lift system. The decreased turbulence allowed the monolayer to remain intact for more than 2 h during the study. The mixing, however, was slow, requiring up to 2 min for complete mixing. The modifications also eliminated edge damage. The cell monolayers were grown directly on Lexan rings with a collagen-coated Nitex mesh support, and the whole ring was inserted into the chamber. Since the cells were grown to confluence, covering the edge of the ring, and since there was no pressure exerted on the monolayer itself, edge damage was avoided.

For the electrical study, 10^6 cells were plated per ring, with a surface area of 1.98 cm². The experiments were carried out between 40 and 56 h after plating. The cell monolayer was rinsed in Ringer solution and then inserted into the chamber. The procedures after this point followed those described by Binder et al. (2). The mucosal and serosal reservoirs contained identical volumes of oxygenated Ringer solution (pH 7.4) at 37°C. The Ringer solution used in these experiments contained (in mM): Na, 140; K, 5.0; Ca, 1.2; Mg, 1.2; Cl, 119.8; HCO₃, 25; H₂PO₄, 2.4; HPO₄, 0.4, and glucose, 10.

The potential difference (PD) across the cell monolayer was measured by calomel electrodes in 3 M KCl and was monitored with a potentiometer. Spontaneous tissue PD was short-circuited and nullified by an automatic voltage clamp with Ag-AgCl₄ electrodes throughout the experiment, except for 5–10 s every 2.5 min while the PD was being recorded. Tissue conductance (G) as calculated from the PD and the short-circuit current (Isc) according to Ohm’s law.

Materials. All the radionuclides were obtained from New England Nuclear, Boston, MA. Peptide hormones used in this study were generously supplied by Dr. Jean Rivier, Dr. Marvin Brown, and Dr. Wylie Vale at The Salk Institute, La Jolla, CA. Prostaglandin E₁ (PGE₁) was purchased from the Upjohn Co., Kalamazoo, MI; carbachol and sodium ricinoleate were purchased from ICN Pharmaceuticals, Plainview, NY; dopamine, serotonin creatine sulfate, and calcium ionophore A23187 were purchased from Calbiochem-Behring, La Jolla, CA; verapamil was obtained from Knoll Pharmaceuticals, Whippany, NJ; clonidine from Boehringer-Ingeheim, Ridgefield, CT; and epinephrine bitartrate from Sigma Chemical, St. Louis, MO. Cholyglycine was a gift from Dr. Alan F. Hofmann, San Diego, CA.

RESULTS

Morphological studies. The T₄₄ cells grew to confluence as monolayers with the basolateral surface attached to the glass, plastic, or collagen-coated nylon mesh and the microvillus membrane facing the media. Tight junctions and desmosomes were present between adjacent cells. Freeze-fracture examination also demonstrated the presence of typical tight-junction strands between cells (Fig. 2). Scanning electron microscopy revealed an abundance of small microvilli on the surface of the T₄₄ cells facing the media. This morphological study demonstrated that the T₄₄ cell line retains structural polarity typical of differentiated epithelial cells.

Functional studies. Confluent cell monolayers maintained transepithelial electrolyte transport and responded to a number of peptide hormones and neurotransmitters. Because changes in the PD followed changes in the Iₛₑ closely, only changes in Iₛₑ are shown; the Iₛₑ is expressed as μA/monolayer (1.98-cm² surface area). Gₑ, as calculated from PD and Iₛₑ, was 10 ± 2 mmho/cm² (mean ± SE, n = 97) and did not change significantly after the addition of various compounds utilized in this study. The relatively high resistance of the cell monolayer corresponded with the large number of tight-junction strands seen on freeze-fracture study (Fig. 2).

The T₄₄ monolayers have receptors for many peptide hormones and neurotransmitters and maintain vectorial electrolyte transport, as indicated by the changes in PD and Iₛₑ. These results are summarized in Table 1 and Fig.
Fig. 2. Electron micrographs of Ts4 human colonic tumor cell line. A: thin-section transmission electron micrograph of a confluent Ts4 cell monolayer grown on a plastic culture dish (×3,000 magnification). B: higher magnification (×15,000) view of a Ts4 cell in monolayer culture showing microvilli and microtubules facing culture media with basolateral surface attached to culture dish. C and D: demonstration of tight junctions and desmosomes between cells (×30,000 magnification). E: scanning electron micrograph of Ts4 cell monolayer showing an abundance of relatively short microvilli (×3,000 magnification). F: freeze-fracture studies reveal typical tight-junctional strands; depth and complexity of tight-junction complex vary from cell to cell.

Table 1. Alteration of short-circuit current induced by other compounds

<table>
<thead>
<tr>
<th>Agents</th>
<th>ΔIsc, µA/monolayer of 10⁶ cells on 1.98 cm²</th>
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<tbody>
<tr>
<td>Cholylglycine, 0.2 mM</td>
<td>23 ± 3 (4)</td>
</tr>
<tr>
<td>Sodium ricinoleate, 1 mM</td>
<td>24 ± 5 (4)</td>
</tr>
<tr>
<td>Bombesin, 1 µM</td>
<td>0 ± 2 (4)</td>
</tr>
<tr>
<td>Neurotensin, 1 µM</td>
<td>0 ± 2 (4)</td>
</tr>
<tr>
<td>Substance P, 1 µM</td>
<td>−2 ± 2 (4)</td>
</tr>
<tr>
<td>Serotonin, 0.1 mM</td>
<td>0 ± 1 (5)</td>
</tr>
<tr>
<td>Met-enkephalin, 1 µM</td>
<td>0 ± 0 (5)</td>
</tr>
<tr>
<td>Epinephrine, 1 µM alone</td>
<td>5 ± 1 (4)</td>
</tr>
<tr>
<td>Epinephrine with 10 µM</td>
<td>1 ± 0 (4)</td>
</tr>
<tr>
<td>propranolol</td>
<td></td>
</tr>
<tr>
<td>Clonidine, 10 µM</td>
<td>0 ± 0 (4)</td>
</tr>
<tr>
<td>Dopamine, 10 µM</td>
<td>3 ± 1 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE for the no. of experiments shown in parentheses. ΔIsc, change in short-circuit current.

3. The Isc is the electrical current required to nullify the PD and can be used as an indicator for alteration of electrolyte absorption and secretion. Since the Isc was not altered by the addition of glucose, it is likely to lack the sodium-glucose symport. The Ts4 monolayers responded to a variety of peptide hormones, neurotransmitters, and other compounds known to stimulate electrolyte secretion and increase Isc (Fig. 3). These include vasoactive intestinal polypeptide (VIP), PGE₁, and carbachol. In addition, calcium ionophore A23187, cholylglycine, and sodium ricinoleate increased the Isc. All these responses were quantitatively and qualitatively similar to their effect in the isolated small and large intestine reported previously (1, 3–5, 8, 10, 11, 13, 14, 17, 18). The effect of VIP was seen at 10⁻¹⁰ M VIP and reached the maximum at about 10⁻⁸ M (data not shown). Bombesin, neurotensin, and substance P had no effect on the Isc. The Ts4 monolayers also responded to somatostatin, an agent known to inhibit electrolyte secretion (6, 7). The somatostatin response by Ts4 cells was observed only when the monolayers had been pretreated with agents that stimulated intestinal secretion, e.g., VIP, PGE₁, and ionophore A23187. Somatostatin by itself had no effect on the Isc. Similar results were obtained with verapamil; it decreased the Isc induced by VIP and PGE₁ but had little or no effect when they were added alone. Met-enkephalin, clonidine, and dopamine had no effect on the Ts4 monolayers. Epinephrine bitartrate slightly increased the Isc; this effect was the opposite of what occurs in the isolated intestine (9, 15). The epinephrine effect was blocked by propranolol.

Discussion

We have characterized the Ts4 cell line, a human colonic epithelial cell line, and demonstrated that it is suitable for electrolyte transport studies.

Morphologically, the Ts4 cells retained many properties of epithelial cells. They grew as a monolayer and maintained the polarity of the cells. The plasma mem-
brane of the monolayers formed microvilli facing the media and formed tight junctions between cells. It should be noted that these properties require the presence of serum. Newborn calf serum was used in our studies. When the cells are grown in serum-free synthetic media (supplemented with insulin, glucagon, epidermal growth factor, transferrin, hydrocortisone, triiodothyronine, selenium, and ascorbic acid), the cells grow approximately three times faster than normal but do not form confluent monolayers. Rather, they form the glandlike structures closely resembling the original tumor (12).

The ability of the cells to form monolayers when grown in serum-supplemented media, where the growth rate is slower, suggests that there are factors in the serum that allow better differentiation of the cells and decrease their growth rate. Development of serum-free synthetic media suitable for growing the T84 cells as a well-differentiated monolayer will be an important step in the future. This suggests either that the monolayer has not been presensitized by VIP or that, in the isolated intestine, VIP may have much difficulty reaching the basolateral membrane’s binding site due to the large molecular size or due to enzymatic destruction of the peptide. c) The effect of somatostatin is seen only when the monolayer is pretreated with secretagogues. In the isolated intestine, somatostatin always has a small effect by itself, although in some systems, such as the rat colon, the inhibition of secretagogue action is much more pronounced than the effect of VIP given at the basal state (7). The results indicate that there are somatostatin receptors on the epithelial cells and also suggest that the effect of somatostatin is to inhibit secretion rather than stimulate an absorptive process unrelated to secretion. d) The effect of carbachol is also interesting in that it was very similar to the effect reported in the isolated rabbit ileum, which was thought to represent both a direct secretory effect on the enterocyte and an opposing effect via adrenergic release (18). Our finding suggests that the sharp increase and decrease in the Isc caused by carbachol is likely to be a result of direct stimulation of the cholinergic receptor on the epithelial cells.

In conclusion, the T84 cell line is a physiologically suitable cell line for electrolyte transport studies. We have demonstrated that these cells retain the structural and functional polarity of transport epithelia. The cells
also respond to a variety of peptide hormones and neurotransmitters in a manner similar to isolated intestine, indicating the presence of receptors and transport processes. Therefore, the cell line should serve as a useful tool for investigating how various peptide hormones or neurotransmitters work and how they affect various electrolyte transport pathways. We hope that this cell line will allow the precise determination of the biochemical processes induced by peptides or neurotransmitters from receptor binding to the activation of various cellular mediators, which finally leads to stimulation of the transport pathways or channels.

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