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Maintenance and Functional Properties of Primary Turtle Bladder Epithelial Cells Cultured on BD Falcon™ Cell Culture Inserts

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Introduction

The purpose of the study was to develop the procedures needed to maintain turtle bladder epithelial cells in primary culture, and to determine if monolayers formed in culture retained ion-transporting functions of that tissue. One of the problems encountered was to find a suitable substrate upon which the cells could be attached and which met the following requirements: (i) optical properties that did not interfere with phase microscopy; (ii) sufficient permeability for ions and molecules; and (iii) mountable in a form convenient for electrophysiological measurements.

Materials and Methods

Cell Isolation and Culture

The epithelial cells lining the turtle urinary bladder were detached by exposing the luminal bladder surface to a Ca, Mg-free turtle Ringers solution containing 1 mM EDTA for one to two hours at room temperature. After trituration, the solution was collected, the luminal bladder surface was rinsed one time with fresh solution and the solutions with cells were pooled. Cells were collected and rinsed several times by low-speed centrifugation.

The final cell pellet was resuspended in Turtle Medium (turtle Ringers solution supplemented with D-glucose, essential and nonessential amino acids, vitamins, hormones and penicillin-streptomycin). Approximately 3×10^6 cells were seeded onto individual BD Falcon™ Cell Culture Inserts (approximately 5 cm² area) that were coated with rat tail collagen. Turtle Medium was added to the well and the cultures were incubated at least four days until confluence was achieved at 30°C in a humidified 5% CO₂ atmosphere. Medium was replenished every four days.

Microscopy

Populations were observed using an inverted, phase contrast microscope. Some cultures were stained using a AgNO₃-UV light procedure to identify potential carbonic anhydrase-rich cells in the population (Durham, 1989)¹ and viewed by bright field microscopy.

Electrophysiologic Measurements

Cultures were mounted between two halves of a custom-designed Ussing-type chamber and bathed on both sides by identical turtle Ringers solutions bubbled with 5% CO₂ and maintained at 30°C. The potential difference across the monolayer was detected using calomel electrodes and the current required to maintain the potential difference across the monolayer at zero (short circuit current; I_{sc}) was passed via Ag-AgCl electrodes connected to the chamber via salt bridges. The electrical resistance across the monolayer was calculated by determining the current required to produce a change of 10 mV across the monolayer. All values are reported for 5 cm² of area, with I_{sc} in uA and R in ohms.

Results and Discussion

Culture and Morphology

Isolated turtle bladder epithelial cells readily attached to and spread out on the filters. Although growth rate was not quantitated, the populations routinely required seven days to attain confluence even though unattached cells were removed after four days, suggesting the cells did proliferate. The cells in primary cultures exhibited morphology similar to the native bladder epithelium. The optical clarity of the membrane was excellent, permitting easy observation of living cells using phase contrast microscopy.

Detection of Carbonic Anhydrase-Rich Cells

Cells maintained on filters were stained using a AgNO₃-UV light procedure which identifies carbonic anhydrase-rich (CA-rich) cells (*Figure 1*).

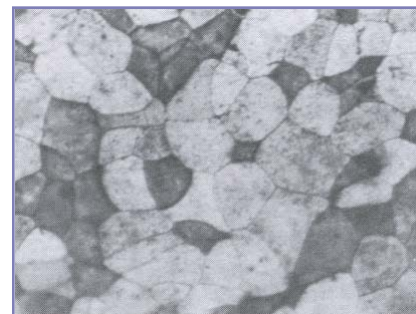


Figure 1: Light micrograph of primary turtle bladder cells cultured on BD Falcon Cell Culture Inserts. Cells were stained by a AgNO₃-UV light procedure, which stains the borders between cells (tight junctions) and a minority cell population possessing high carbonic anhydrase activity.

continued

Following the staining procedure, the populations were observed using bright field microscopy. The borders between the cells (tight junctions) were clearly visible and strongly stained, as had been observed in the intact bladder, and some cells were strongly stained while others were not. The intact bladder cells which stain heavily with Ag exhibit high CA activity (Durham, 1989).¹ Although we have not colocalized Ag staining and CA activity in the primary cultures, the presence of cells staining both weakly and strongly with Ag in these populations supports the idea that the populations contain both granular (G) and CA-rich cells, respectively.

Electrophysiology

In the intact bladder, it has been demonstrated that the major component of positive short circuit current (I_{sc} ; positive charges flowing from the luminal to serosal sides) is due to Na reabsorption from the luminal fluid. In the intact bladder this I_{sc} is inhibited by micromolar concentrations of amiloride added to the luminal solution, or ouabain added to the serosal solution. We examined the electrophysiological properties of primary cultures of turtle bladder epithelial cells to determine if they have maintained both the transport properties and polarity required to produce this Na transport function (Figure 2 and Table 1).

Condition	Resistance (ohms-5 cm ²)
No filter	15
Filter alone	160
Filter with cells	2147 ± 480 (n=2)

Table 1: The resistance across: (1) bathing solutions alone (no filter); (2) filters and; (3) filters with monolayer of cells.

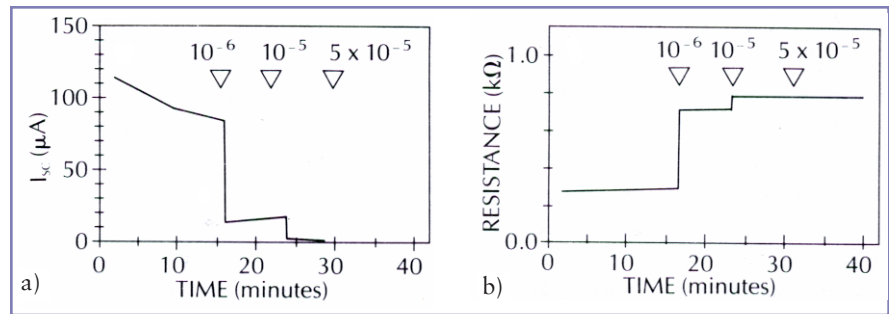


Figure 2: Measurement of short circuit current (a) and resistance (b) of confluent populations of turtle bladder epithelial cells maintained on BD Falcon Cell Culture Inserts. Populations were mounted in a modified Ussing chamber between symmetrical turtle Ringers solutions. Short circuit current (I_{sc}) and resistance were measured under voltage clamp conditions. Values shown are respective molar concentrations of amiloride added to apical solution.

The resistance of the turtle Ringers solution in the chamber was 15 ohms (Table 1). The collagen-coated filters increased the resistance to 160 ohms. After a monolayer of cells from the bladder had grown on the filters, the resistance was substantially higher, approaching 2,000 ohms. These data demonstrate that the filters have an intrinsically high permeability to ions which allow for the determination of electrical parameters across cells attached to the filters.

In a representative experiment (Figure 2) the I_{sc} initially 120 μ A, declined slowly over a 20 minute period. Addition of 1 μ M amiloride to the luminal solution simultaneously decreased I_{sc} and increased resistance in less than one minute. Increasing the amiloride concentration to 10 μ M and then to 50 μ M produced progressively smaller decreases in I_{sc} , approaching 0 μ A. The addition of amiloride increased the resistance of the monolayer by greater than two-fold, demonstrating that amiloride inhibits a conductive pathway (Na channel). Addition of amiloride to the serosal solution did not affect I_{sc} , but addition of 10 μ M ouabain to this solution inhibited I_{sc} over a time course of

approximately 30 minutes. These data demonstrate that cultured turtle bladder epithelial cell monolayers possess a transepithelial net Na flux which is comprised of a luminal, amiloride-inhibitable Na channel and a serosal ouabain-inhibitable Na-K-ATPase, as has been shown in the intact bladder.

It can be concluded that: (i) the bladder's Na-reabsorptive function and pharmacological characteristics can be maintained in monolayers of primary cultures of the bladder epithelial cells; (ii) the tight junctions and luminal and serosal polarity of the epithelial cells are also maintained when grown on these filter supports; and, finally, (iii) the filter supports are of sufficient permeability to ions and small molecules to allow for nutrient and chemical access to the membranes of the cells attached to the filter surface.

Reference

- Durham, J.H., *Changes in urinary epithelial cells during adaptation to acid-base imbalance*. *Kid, Int.* **35**:453 (1989).

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