The thyroid cell monolayer in culture
A tight sodium absorbing epithelium

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Abstract. When cultured on collagen coated nitrocellulose filters, thyroid cell monolayers form morphologically and functionally polarized monolayers. The bioelectric parameters of these monolayers were measured after mounting in Ussing chambers; transepithelial potential (V_{ab}), short circuit current (I_{sc}), and transepithelial resistance were respectively 12 ± 1 mV (apical side negative), 3.8 ± 0.2 μA cm^{-2} and 3250 ± 214 Ω cm^{2} (mean ± SEM, n = 75). Eighty two percent of the short circuit current was related to sodium absorption as shown by inhibition by apical amiloride (K_{0} = 0.2 μM) and by basal ouabain (K_{1/2} = 0.3 μM). Amphotericin B (5-25 μg/ml) added to the apical bath increased I_{sc} suggesting a apical rate-limiting step. Step by step replacement of choline by Na^{+} in a Na^{+}-free medium resulted in a progressive increase in V_{ab} and I_{sc} with half maximal effect at 20 ± 1 mM Na^{+}. Thyrotropin (TSH) increased I_{sc} and V_{ab} in a biphasic way with a transient maximum after 5 min and a plateau after 20 min (about four times the basal level at 100 μU/ml TSH). This increase in sodium transport was also inhibited by apical amiloride. Thus, in culture, the thyroid cell monolayer behaves as a tight sodium absorbing epithe-lium with a rate limiting apical amiloride sensitive Na^{+} entry mechanism and a basolateral Na^{+}, K^{+}-ATPase as the electromotive force. The transepithelial Na^{+} current is stimulated by thyrotropin.

Key words: Thyroid cell — Monolayer — Na^{+} transport — Amiloride — Amphotericin B — Na^{+}, K^{+}-ATPase — Thyrotropin

Materials and methods

Cell culture. Porcine thyroid cells were isolated by discontinueous trypsin-ethyleneglycol-bis(β-aminoethyl ether)N,N,N',N'-tetra acetic acid (EGTA) treatment as previously described [20]. Isolated cells were suspended in Eagle’s Minimum Essential Medium supplemented with non-essential amino acids, 10% newborn calf serum, penicillin (50 U/ml) and streptomycin sulfate (50 μg/ml). Five Millipore filters (HAFT, 0.45 μm, 25 mm diameter) were attached with a soldering iron to the bottom of 100 mm diameter Falcon tissue culture dishes and then coated with collagen (Seromed, Munich, FRG). Isolated thyroid cells were seeded at a density of 2 x 10^{6} cells/ml with 15 ml per dish. Cells were incubated at 37°C in a 5% CO_{2}, 95% air, water saturated atmosphere. Four days after seeding, the filters were released and transferred upside down into Falcon 35 mm diameter dishes containing 2 ml of culture medium. The medium was changed routinely every third or fourth day. Monolayers were used between the 7th and 21st day.

Light microscopy. Cell monolayers on filters were fixed in 1.2% formaldehyde in calcium and magnesium free Dulbecco’s phosphate buffered saline (PBS), then postfixed in a 1/9 mixture of 40% formaldehyde in water and 95%
ethanol for 30 min at 37°C, rinsed in PBS and stained for 30 min at 37°C in a 1% toluidine blue solution in 26 mM sodium borate. The stained filters were rinsed in PBS and dehydrated in graded ethanol. The dehydrated filters were incubated in xylene until they became translucent and then mounted between a glass slide and a coverslip in Eukitt (Vitromed, Basel, Switzerland).

Morphometric measurements. A simple square lattice system (test area 96 cm²; number of test points 24; distance between test points 2 cm) was used to estimate the morphometric parameters at a final magnification of 238. The cell density was estimated following the forbidden line counting rule [28]. For the cell neighbouring determination, sampled cells were chosen if covered by one lattice point. Finally, the linear density of cell junctions (La) was determined from the number of intersections of junctions with the horizontal lines of the lattice (N1) using the relation $L_a = N_1 \pi / 2$ [28].

Electron microscopy. Cell monolayers were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 10 min at 37°C, then for 1 h at room temperature and postfixed in 2% OsO₄ for 1 h, dehydrated in ethanol, and embedded in Epon. Thin sections were cut perpendicular to the filter and stained with uranyl acetate and lead citrate.

Electrical measurements. Thyroid monolayers on collagen coated filters were introduced between two Ussing half chambers (volume = 1.5 ml, window area = 1.54 cm²) as described by Grasset et al. [13]. To reduce edge damage, the filters were mounted between two rings of silicone paste (Rhodorsil, Rhône-Poulenc, Paris, France). Each compartment of the chamber was connected to a reservoir with thermostatic (37°C) and gaseous control. A bicarbonate-Ringer solution (in mM: NaCl 117.6, NaHCO₃ 20, KCl 5, Na₂HPO₄ 2.4, KH₂PO₄ 0.6, MgCl₂ 1.6, CaCl₂ 1.6, glucose 10) was used as apical and basal bathing solutions. The Na⁺ free solution had the following composition (in mM): choline chloride 117.6, choline bicarbonate 20, K₂HPO₄ 2.4, KH₂PO₄ 0.6, MgCl₂ 1.6, CaCl₂ 1.6, glucose 10. A pH of 7.4 was obtained by controlled bubbling of O₂-CO₂ (93%-7%).

Transepithelial short circuit current ($I_{sc}$) and potential difference ($V_{ab}$, basal side as reference potential) were monitored by an automatic current-voltage clamp (World Precision Instrument, New Haven, CT, USA) that compensates for the electrical resistance of the fluid. Current-voltage curves were obtained by passing 10 – 50 µA bipolar current pulses (500 ms) and measuring the resulting changes in $V_{ab}$. Dose response curves for effectors were obtained as follows: the effector dissolved in the incubation medium was added to the bathing buffer (apically or basally) and $I_{sc}$ was allowed to reach a new steady state after which more drug was added to produce a second steady state and so on, until a near maximal effect was obtained.

Results were expressed as mean ± SEM for $n$ independent experiments. Student’s t-test was used although the