Electron Microprobe Analysis of the Different Epithelial Cells of Toad Urinary Bladder

Electrolyte Concentrations at Different Functional States of Transepithelial Sodium Transport

Roger Rick*, Adolph Dörge, Anthony D.C. Macknight**, Alexander Leaf***, and Klaus Thurau

Department of Physiology, University of Munich, Pettenkoferstr. 12, D-8000 Munich 2, Germany

Received 25 April 1977

Summary. The electrolyte composition of toad urinary bladder epithelial cells has been measured using the technique of electron microprobe analysis. Portions of hemi-bladders, which had been mounted in chambers and bathed with a variety of media, were layered with albumin solution on their mucosal surfaces and immediately shock-frozen in liquid propane at -180 °C. From the frozen material 1–2 μm thick cryosections were cut and promptly freeze-dried for 12 hr at -80 °C and 10^-6 Torr. Electron microprobe analysis using a scanning electron microscope, an energy dispersive X-ray detector, and a computer programme, to distinguish between characteristic and uncharacteristic radiations, allowed quantification of cellular ionic concentrations per kg tissue wet wt by comparison of the intensities of the emitted radiations from the cells and from the albumin layer. Granular, mitochondrial-rich, and basal cells, and the basal portions of goblet cells, showed a similar composition, being high in K (about 110 mM/kg wet wt) and low in Na (about 13 mM/kg wet wt). The apical portions of goblet cells were higher in Ca and S and lower in P and K, presumably reflecting the composition of the mucus within them. With Na-Ringer's as the mucosal medium, cells gained Na and lost K, when their serosal surfaces were exposed to ouabain, 10^-2 M. Replacement of mucosal Na by choline virtually prevented these ouabain-induced changes. Cellular ion contents were unchanged when Na in the serosal medium was replaced by choline. No differences in Na and K concentrations were detected between nuclei and cytoplasm. These results provide independent support for the hypothesis that the cellular Na transport pool in toad bladder epithelial cells derives exclusively from the mucosal medium and that no important recycling of Na occurs from the serosal medium to the cells.

* Present address: Department of Physiology and Biophysics, University of Miami Medical School, Miami, Florida 33152.
** Permanent address: Department of Physiology, University of Otago Medical School, Dunedin, New Zealand.
*** Permanent address: Departments of Medicine, Massachusetts General Hospital and the Harvard Medical School, Boston, Massachusetts 02114.
Amphibian epithelia such as frog skin and toad urinary bladder have been extensively studied in an attempt to understand their role in the transport of solutes and water. In particular, the active transport of Na by these tissues has been a focus of investigations. Since, even across the histologically simple toad bladder, several possible pathways exist by which Na ions can be reabsorbed from urine to body fluids (from mucosal to serosal bathing media), much attention has been directed to the route of active transepithelial transport. Potential pathways are either intercellular spaces with Na penetrating the limiting junctions and never entering cells, or transcellular routes with Na passing through the cells. Since Na from the mucosal medium has been shown to enter the mucosal layer of cells [12] and transepithelial Na transport was found to be coupled to cellular energy metabolism [10], it is most likely that active transport of Na follows a transcellular rather than an intercellular route. However, with several different cell types present in the mucosal layer of the epithelium, it is possible that one or more types may be engaged in transepithelial Na transport. One interpretation of the finding that only a fraction of the cellular Na is exchangeable from the mucosal side [13] might be that only a certain cell type is involved in transepithelial Na transport.

In the present study cellular electrolyte concentrations in the different epithelial cell types of toad urinary bladder under varying conditions of transport were measured using electron microprobe analysis in an attempt to identify the epithelial cell types involved in transepithelial transport of Na and to define the magnitude of the cellular Na transport pool.

**Materials and Methods**

Female toads of the species *Bufo marinus* (Dominican Republic, National Reagents, Bridgeport, Conn.) were kept in plastic troughs with free access to tap water. Toads were pithed and their urinary bladders dissected and cut into 4–5 pieces. The pieces were then mounted on lucite rings (exposed surface area 3.2 cm²), which were inserted into Ussing-type chambers. The experiments were performed under short-circuited conditions, using an automatic voltage clamping device. Transepithelial potential difference (PD) was measured every 30 min for 2 min. During the preincubation period the bladders were incubated on both the mucosal and serosal sides in Ringer’s solution for some 60 min until the short-circuit current had reached a steady-state value. After preincubation the half chambers were emptied and refilled with new incubation solutions, according to the following protocol:

a) Control: both sides with Ringer’s solution

b) Ouabain: both sides with Ringer’s solution, serosal medium containing $10^{-2}$ M ouabain