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Mitochondria-rich cells in amphibian skin epithelium: Relationship of immuno- and peanut lectin labeling pattern and transport functions

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This paper is dedicated to the dear memory of Professor C Barker Jorgensen (1915—2007) of Copenhagen

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Ion transport;
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Band 3;
H^+-ATPase;
Anurans;
Urodeles;
Na^+ transport

Summary

Mitochondria-rich cells are an integral component of the epidermis of amphibian skin and play a functional role. Whereas the principal cell compartment of the epithelium is specialized almost exclusively for active uptake of sodium, the mitochondria-rich cells perform other diverse ion-transport functions, including transport of Cl^-, H^+, HCO_3^- and organic molecules. These transporting functions differ in different species. Antibodies, such as those directed against band 3, H^+-ATPase, and also peanut lectin (PNA), bind specifically to the mitochondria-rich cells, but do so differently in various species. Examination of these immunolocalizations and lectin labeling in the skin of over 10 amphibian species, including both Anurans and Urodeles, illustrate species-specific differences. The binding pattern and the transport capabilities of the skin in the various species do not show a universal correlation, they appear to be species specific and do not permit construction of a general scheme common to all the species studied. The mitochondria-rich cells of heterocellular epithelia and their roles in ion transport remain a subject that requires further studies to elucidate their particular functions within the framework of the whole epithelium.

Introduction

The epidermis of the amphibian skin, in addition to its protective function, provides an active barrier with selective permeability to water and

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ions. It is influenced by environmental conditions and is finely controlled by hormones. The stratified epithelium is composed mainly of two cell types in addition to the tubular secretory ducts of glands. The major cell type, the principal cells, are interconnected through gap junctions (Farquhar and Palade, 1964), forming a functional cellular unit, which is specialized in the active uptake of Na^+. This function of the epithelium has been studied extensively and thoroughly reviewed (Kristensen and Ussing, 1992; Katz and Nagel, 1994; Nagel and Katz, 2003). The other epithelial cells in the epidermis, the mitochondria-rich (MR) cells, are positioned between the principal cells at the outer aspect of the epithelium; they comprise only a small moiety of the epithelium. The MR cells, also known as flask cells (Whitear, 1975), label specifically for the binding of various antibodies and lectins, and differently in various amphibian species. Specific functions including: Cl^- conductance, H^+, HCO_3^- transport and secretion of organic molecules, have been assigned to these cells, but differ among species (Larsen, 1991; Katz and Nagel, 1994; Ehrenfeld, 1998). Based on ion replacements, hormones and application of specific inhibitors, as well as direct measurements that preclude a finite Cl^- conductance in the principal cells (Nagel, 1989; Larsen, 1991), it is clear that Na^+ and Cl^- transport across the epithelium must be cellularly separated and executed by different cells. However, no common association has been established with regard to particular transport functions and structural features of the MR cells among the various amphibian species (Brown et al., 1981). Having studied the transport functions of skin epithelium in relation to whole epithelial composition in over 10 species of amphibians, including both Anurans and Urodeles, it was desirable to analyze the structural features of MR cells in correlation with the transport properties of the skin. This should elucidate questions regarding the enigmatic function of these cells in the epidermis of amphibian skin.

Material and methods

Animals

Bufo viridis, *Bufo regularis*, *Rana ridibunda*, *Pelobates syriacus*, *Hyla arborea*, *Salamandra salamandra and Triturus cristatus* were collected locally in the Haifa area. *Bufo bufo* were from England, *Bufo marinus* originated from the Dominician Republic and *Rana pipiens* from Virginia (USA). *Necturus, Ambystoma* tygrinum and *Xenopus laevis*, Daudin (South African origin) were obtained from commercial dealers. Only adult forms were used in the present study. The animals were kept in the laboratory in tap water or had free access to water (toads) at room temperature (approx. 21 °C); they were fed mealworms once a week. Maintenance and handling of the animals were conducted according to protocols approved by the local institutional ethical committee. Animals were used following a minimum of 10–15 days of laboratory acclimation.

Tissue samples, preparation and labeling

Pieces of skin were obtained from animals anaesthetized with MS-222 (tricaine methanesulfonate) administered orally with a spatula), and were fixed in buffered Bouin's fixative overnight for immunohistochemical and lectin labeling. After dehydration in a series of increasing ethanol concentrations, samples were embedded in paraffin wax, and sectioned (25–30 μm thick) for confocal microcopy (see below).

Fresh skin pieces were silver stained, as described previously (Katz et al., 2000). Briefly, the skin pieces were immersed in 0.25% $AgNO_3$ for 2 min, washed and illuminated for 30 min by strong light.

Isolated epithelia were obtained after treatment with collagenase (from *Clostridium histolyticum*, Sigma-Aldrich) 1 mg/ml for 60–90 min; the isolated epithelia were exposed to 0.05% methylene blue (Sigma-Aldrich) on the serosal side for 5–10 min, and were then optically sectioned (Zeiss IM35 inverted microscope equipped with 63 × objective and Nomarski (DIC) optics). The use of the two staining methods (silver staining and methylene blue incubation) permits identification of the MR cells within the whole epithelium, both the outer surface and the whole cell.

For transmission electron microscopy, tissue pieces were incubated in 2.5% glutaraldehyde and 0.1 M Na-cacodylate buffer, pH 7.4, postfixed in 2% OsO_4 in 0.1 M Na-cacodylate buffer, dehydrated in a graded series of ethanols, and embedded in Epon 812. Ultrathin sections were contrast stained with uranyl acetate and lead citrate and examined in a Zeiss EM9 electron microscope at 80 kV.

An indirect immunohistochemical technique was used to localize band 3 and H^+-ATPase in skin epithelium. Anti-band 3 polyclonal antibody was raised in our laboratory against band 3 of human erythrocytes (Devuyst et al., 1993), and anti-70 kDa segment of H^+-ATPase monoclonal antibody was