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The voltage-dependent chloride current conductance of toad skin is localized to mitochondria-rich cells

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The chloride current across the isolated epithelium from saline-acclimated *Bufo viridis* toads was studied using the extracellular vibrating probe technique. Local peak current densities varying between 5 and 100 $\mu A/cm^2$ were recorded over subpopulation of mitochondria-rich cells, but never over granulosum cells. These local transepithelial currents had characteristics similar to the activated chloride current observed in the whole skin (Katz, U. and Larsen, E.H. (1984) J. Exp. Biol. 109, 353–371). Replacement of the apical Ringer with chloride-free (nitrate) ringer resulted in reversible reduction in the current at the mitochondria-rich cells. It is concluded that the mitochondria-rich cells are the principal site of passive chloride conductance across the epithelium.

Amphibian skin epithelium is endowed with NaCl uptake mechanism which transports sodium actively against its electrochemical gradient [1]. Chloride was believed to follow passively via extracellular path. Recent accumulated evidence suggests, however, a cellular pathway for chloride movement, separate from the major granulosum cells of the epithelium [2]. Since the evidence was indirect, we decided to employ the vibrating probe technique [3] which provides a direct method for measurement of local electrical current on the toad skin epithelium. We show that chloride-dependent peak current densities are localized over mitochondria-rich cells of the epithelium.

A chloride-selective conducting pathway has been characterized in amphibian skin [1] with rectifying properties similar to the Hodgkin and Huxley [4] model for excitable membranes, but with slower time-course [2]. It has also been found that the chloride conductance of the whole skin is correlated with the number of mitochondria-rich cells [5–7]. These cells which are 5–15 μm in diameter comprise about 10% of the outer epithelial cell layer, and contact the apical side.

Split epithelium was obtained from *Bufo viridis* skin after digestion with collagenase (0.4 mg/ml) at room temperature according to Rajerison et al. [8]. The isolated epithelium which is transparent was mounted in a horizontal Ussing chamber bathed with normal Ringer solution (containing in mmol/l: Na^+ 120, K^+ 3, Ca^{2+} 1, Mg^{2+} 0.5, Cl^- 125, Tris buffered pH 7.8) on both sides, and viewed by an inverted Zeiss (IM35) microscope. The mitochondria-rich cells are easily and distinctly visualized in this way, and the vibrating probe (gold plated and platinized) with tip diameter of 3–5 μm which is approaching near vertical, was vibrated at frequencies of 300–900 Hz [9]. Fig. 1 shows current records by the vibrating probe during a scan of the apical surface of the epithelium from saline acclimated (115 mmol/l NaCl for 2 weeks) toad. The tissue was clamped to −100 mV (outside negative) and was allowed 2

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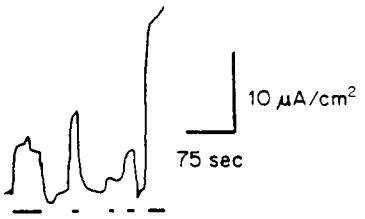

Fig. 1. Current density measurements by a vibrating probe over the apical surface of a toad skin epithelium clamped to 100 mV (outside negative). The bars bellow the curve show when the probe (about 25-35 μm above the tissue) was positioned over a mitochondria-rich cell.

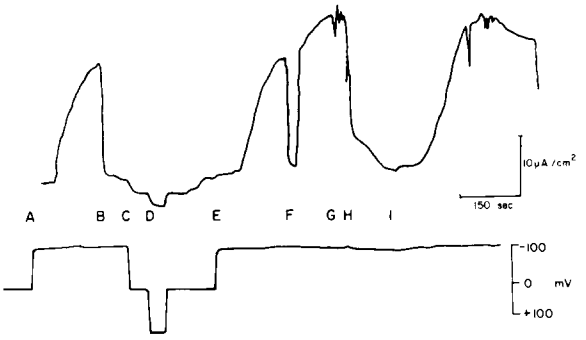

Fig. 2. Vibrating probe-measured current at a mitochondria-rich cell in the toad skin epithelium. Upper trace: current recorded over a mitochondria-rich cell. Lower trace: clamping manouvers across the whole tissue. From left to right: the probe was positioned over a mitochondria-rich cell under short-circuit (0 mV) conditions. At 'A' the transepithelial potential was clamped to −100 mV (outside neative) and transcellular current is activated. At 'B' the probe was moved 30 μm away from the mitochondria-rich cell and over a granulosa cell, resulting in loss of current. Short-circuiting here to 0 mV ('C') did not have a significant effect on the current. The probe was positioned again over a mitochondria-rich cell and the tissue was clamped in the opposite direction (to +100 mV; 'D') with small current response in the reverse direction. At 'E', the tissue was clamped again to −100 mV with the probe at the mitochondria-rich cell, resulting in current activation. Moving the probe 25 μm aside and back resulted in reversibly diminished current ('F'). At 'G' the upper bath (apical side of the epithelium) perfusion began, and at 'H' the chloride-Ringer was replaced by nitrate, resulting in diminished current. With the probe held at the same position, chloride-Ringer was resumed at 'I' followed by recovery of the current.

min to fully activate the chloride conductance [6]. Outward peaks current density (corresponding to either positive ions leaving or negative ions entering the epithelium) of 5 to over 20 $\mu A/cm^2$ were observed over a subpopulation of mitochondria-rich cells. The proportion seemed to vary between preparations: 30% to 90% were our crude estimates in five preparations, although no quantitative study was made that would allow us to make precise statements about this. The variability in the peaks of current density should reflect the cellular level of control at the apical membrane of the mitochondria-rich cells, while in the long term there is a considerable change in the number of cells [6]. When peaks of current were observed over openings of mucous glands they were separate from the mitochondria-rich cells and their polarity could be reversed with the potential. The spatial resolution of less than 10 μm allowed recording from a single mitochondria-rich cell to be made. Fig. 2 shows that the characteristics of the peak current at a single mitochondria-rich cell share the properties of the activated chloride conductance pathway as described by Katz and Larsen in this species [6]. The time-course of the current activation was the same as for the whole skin. Clamping the skin in the reverse direction resulted in a small non-activated current response ('D') and chloride replacement by nitrate at the apical side diminished the activated current reversibly ('H', 'I'). Extracellular pathway for the chloride conductance around the mitochondria-rich cells seems to be excluded, since it would not show the behaviour of current rectification, which characterizes this system [6].

The apical membrane of the granulosum cells in the amphibian skin epithelium is selectively permeable to sodium ions [10] and these cells behave as a functional syncytium in the transport of sodium across the skin [11]. The intracellular chloride concentration of these cells is above the electrochemical equilibrium [12], which is achieved through a furosemide blockable co-transport system of NaCl at the basolateral membranes and a low chloride permeability. This would aid to the electrochemical driving force for the sodium entry into the granulosum cells. The intracellular chloride concentration of the mitochondria-rich cells on the other hand, is nearly at chemical equilibrium [13], and chloride can therefore move selectively through these cells [14,15], when the electrochemical forces favour it [2]. The data presented in this paper support this hypothesis and show that the activated chloride current conduc-

tance [6] is localized to the mitochondria-rich cells of the skin. A similar conclusion was reached on the basis of pharmacological evidence [1] and more recently in frog skin [14] based on volume measurements.

Cellular heterogeneity appears to be a common feature of many tight epithelia. It has already been observed in toad and turtle urinary bladders [16,17], in the renal distal tubule and the collecting duct [18], as well as in the low resistance opercular epithelium of the fish [9]. The mitochondria-rich cells are separated morphologically and functionally from the granulosum cells in frog skin and in the toad urinary bladder epithelia [11]; they are characterized also by their silver stainability [19] and are the concentrated sites for the carbonic anhydrase of the whole epithelium [20]. These cells provide a cellular shunting pathway for the movement of chloride and other ion species, such as protons or bicarbonate which participate in the acidification or alkalinization of the urine [16,17]. These selective pathways can function either in absorption or in secretion, and they may be passive such as in the amphibian skin, or active such as in the fish opercular epithelium [9] or the turtle and toad urinary bladders [16,17]. However, while it is possible to formulate the functional interrelations of the sodium and chloride permeabilities in the toad and frog skin [2], the significance of the separation of the ion permeabilities in the other epithelia remains to be explained.

The classical epithelial model, which was so instructive in the study of the mechanism of transepithelial sodium transport has in fact been revised recently [1], to integrate the functional cellular heterogeneity of the epithelial organization.

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