The Intracellular Electrical Potential Profile of the Frog Skin Epithelium

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Summary. The potential profile of the frog skin epithelium incubated in Cl-Ringer was reinvestigated with improved technique. Under open circuit conditions (PDtr up to 120 mV) the potential profile was demonstrated to be trough like in contrast to the stair-step like potential profile reported by previous investigators which has probably been recorded after injuring of the punctured cells.

In 67 successful impalements the potential difference across the basolateral membranes was 108 ± 2 mV. The potential across the outer, epithelial-facing membrane was inversely related to the trans-epithelial PD, but was found to be negative (with respect to the epithelial bathing solution) in all punctures. Electrogenic sodium transport might be responsible for part of the potential difference across the basolateral membranes which correlated directly with the short circuit current. The intracellular potential in the short circuited state was -73 ± 2 mV and decreased with increasing short circuit current. It is suggested that these changes result from variations of the outer membrane sodium conductance.

Key words: Frog skin — Potential profile — Microelectrode measurement.

INTRODUCTION

The electrical potential profile of amphibian skin epithelium has been measured repeatedly since 1957 (Engbaek and Hoshiko, 1957; Ussing and Windhager, 1964; Whittembury, 1964; Cereijido and Curran, 1965; Biber et al., 1966; Biber and Curran, 1970; Rawlins et al., 1970; Hvid-Larsen, 1973). From these investigations it is generally accepted that in the open circuited (o.c.) skin, two potential steps exist, both of approximately equal magnitude and positive sign with respect to the epithelial bathing solution. In accordance with the model considerations of Koefoed-Johnsen and Ussing, 1958; the potential step between epithelial bathing solution and cell interior was considered to be due to a sodium-diffusion potential whereas the potential difference between cell and corial bathing solution should represent a potassium diffusion potential, since electrode-like changes in potential were observed after variations of the epithelial [Na⁺] and the corial [K⁺], respectively. However, estimations of the electrochemical gradient across the epithelial membrane revealed that on the basis of the observed values, at least under conditions of low epithelial [Na⁺], passive uptake of sodium at the outer border is not possible unless complex compartmentalisation of sodium during the passage through the epithelium is assumed (Cereijido and Rotunno, 1968). Until now no proof exists for this possibility.

In the short circuited (s.c.) state, intracellular potentials of -15 to -45 mV have been observed. These potentials are small compared to intracellular potentials of other cells but since the sodium permeability of the outer border allows high sodium influx, a low intracellular PD might be predicted from Goldman’s equation. Reduction of the sodium influx should then increase the intracellular potential towards the potassium equilibrium potential. This, however, was not observed (Cereijido and Curran, 1965; Biber et al., 1966).

Almost all previous investigations report that negative potentials (with respect to the epithelial bathing solution) are recorded in outer layers of the skin in the o.c. state. Such measurements were disregarded since no stable values could be obtained. They supposedly represented artifacts produced during the penetration of the microelectrode tip through the
dense layer of the stratum corneum. Recent microelectrode investigations demonstrate, in fact, that potentials of remarkable magnitude can be observed, when the microelectrode tip is situated in the cornified layer (Nunes and Lacaz Vieira, 1975). On the other hand, instability might indicate that the microelectrode injured the small punctured cells, leaving only part of the real intracellular potential. If the basolateral membrane is affected, potentials recorded under o.c. conditions will be strongly influenced by the trans-epithelial potential difference.

From previous investigations it is not clear which criteria were used to insure intactness of the punctured cells. Stability of the observed potentials for more than 20 s, as generally used, is of questionable value since stability is even more likely achieved at a reduced potential level. Therefore, the purpose of the present investigation was, to reinvestigate with improved technique the origin and nature of the first negative potential well within the frog skin. Simultaneous measurement of potential values and the resistance of barriers penetrated by the microelectrode tip demonstrate that the intracellular space of the epithelial cells of the frog skin is negative with respect to the outer solution even under s.c. conditions and that previous measurements are artifacts probably due to damage of the cell membranes.

METHODS

The measurements were performed on the abdominal skin of *R. temporaria* and *R. esculenta*, mounted in a lucite chamber, which enabled the skin to be punctured from the epithelial side perpendicular to the surface (Fig. 1). The skin surface could be observed through a window in the chamber with a binocular microscope. The skin (effective area: 0.75 cm²) was supported by a copper grid at the corial side. To avoid edge damage, silicone high vacuum grease (Merck AG, Germany, No. 7922) was used to seal the edges. Two calomel electrodes for measurement of transepithelial PD (PDₚₑ) were connected via Ringer bridges to both sides of the skin. The bridges ended with an approximate distance of 0.5 mm from the skin. Circular Ag wires coated with AgCl, with a distance of 5 mm from the skin, served as electrodes to apply current for short circuiting the skin (I₀) from an automatic clamping device. PDₚₑ and I₀ were measured alternately for periods of 5–10 s and recorded on one channel of a chart recorder (Servogor, Fa. Metrawatt). The frog skins were incubated in Ringer's solution (110 mM NaCl, 2.5 mM KHCO₃, 1 mM CaCl₂, 5 mM glucose, pH 8.1). The epithelial half chamber (total volume: 0.4 ml) was continuously perfused at a rate of 20 ml/min. The corial half chamber (total volume: 0.3 ml) was perfused at a rate of 10 ml/min by application of a slightly negative hydrostatic pressure, which simultaneously served to attach the skin to the supporting grid.

Microelectrodes with short gently sloping tips (diameter ~ 0.5 μ) were prepared of capillary glass (1.6 mm o.Ø, 1.3 mm i.Ø, Plowden and Thompson, Ltd. Stourbridge, U. K.) on a horizontal puller. After filling with 3 M KCl according to the method of Tasaki et al. (1954), or by pressure filling from behind the microelectrode input resistance (Rᵢₑ) was 5–9 MΩ and tip potentials were below 5 mV. Microelectrode potentials were measured with respect to the epithelial bathing solution using a cathode follower with negative capacity compensation and current injection (H. Ehrler, Homburg, Germany) and were registered on the second channel of the chart recorder. For measurement of Rᵢₑ square wave pulses of 1 nA, 250 ms duration and at 0.2–0.5 Hz, triggered by a Grass stimulator SD 5, were passed through the microelectrode. The microelectrodes were mounted in a special lucite holder on a step motor drive (Narishige Scient. Instr. Lab., Japan) for movement in steps of 1 μ. The electrode position was indicated on a scale and noted, relative to the first contact with the skin surface. Measurements were only accepted when, in addition to criteria which will be described in detail, alterations of Rᵢₑ and tip potential were less than 1 MΩ and 4 mV before and after impalement.

Owing to the rhythmical change between o.c. and s.c. state of the skin, the following potentials can be read from the recording: The intraepithelial potential under s.c. condition (PDₑₛ), the potential difference between the epithelial bathing solution and the microelectrode under o.c. condition (PDₑₒ) and the potential differ-