Intracellular Voltage of Isolated Epithelia of Frog Skin

Apical and Basolateral Cell Punctures

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ABSTRACT Isolated epithelia of frog skin were prepared with collagenase, and the cells were punctured with intracellular microelectrodes across their apical (outer) and basolateral (inner) surfaces. Regardless of the route of cell puncture, the intracellular voltage (V_o^{sc}) in short-circuited isolated epithelia was markedly negative, averaging −70.4 mV for apical punctures and −91.6 mV for basolateral punctures. As in intact epithelia, amiloride outside caused the V_o^{sc} to become more negative (means of −96.7 and −101.8 mV), with a concomitant increase in the resistance of the apical barrier. Increasing the $[K]_i$ of the basolateral solution from 2.4 to 8.0 or 14.4 mM caused rapid step depolarization (5–10 s) of the V_o^{sc} under transepithelial Na transporting and amiloride-inhibited conditions of Na transport, with the ΔV_o^{sc} ranging between 23.9 and 68.3 mV per decade change of $[K]_i$. The finding that the V_o^{sc} of isolated epithelia of frog skin is independent of the route of cell penetration is consistent with the notion that the cells of the stratified epithelium are electrically coupled (functional syncitium). Moreover, the isolated epithelium can serve as a useful preparation, especially in studies designed to investigate the properties of the basolateral surfaces of cells.

Several recent microelectrode investigations of frog skin (see Helman and Fisher, 1977*a*; Nagel, 1976*a*) have been carried out by impaling the apical membranes of the epithelium. When this route is used, the microelectrodes must, by necessity, move through the stratum corneum before they reach the living cell layers located further inside the epithelium. It has been argued that the passage of the microelectrode through the s. corneum leads to major errors in the potential values recorded when the tip reaches the living cells (Nelson et al., 1978). To determine whether such artifacts are indeed important sources of error, we compared measurements obtained by impaling the epithelium through the apical and the basolateral surfaces. The latter route was feasible because we employed epithelia whose underlying connective tissue had been removed by an enzymatic treatment (Aceves and Erlij, 1971).

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METHODS

The procedure used to isolate epithelia was similar to methods originally described by Aceves and Erlij (1971). Abdominal skins of *Rana pipiens berliendieri* (southern frogs, Southwestern Scientific Co., Tucson, Ariz.) were isolated, and after the inner surface of the tissue was gently scraped to partly remove the tela subcutanea, the skins were mounted as flat sheets between chambers (13 cm^2) and bathed with Ringer's solution consisting of 100 mM NaCl, 2.4 mM $KHCO_3$, 2.0 mM $CaCl_2$, and 11.1 mM glucose. Crude collagenase (0.4 mg/ml) was added to the basolateral solution, and skins were incubated at room temperature for 3 h. After testing enzymes from several sources and at various concentrations and times, we found that the open-circuit voltage and short-circuit current during incubation with 0.4 mg/ml of collagenase (CLS II, Worthington Biochemical Corp., Freehold, N. J.) did not differ significantly from the spontaneous changes recorded in untreated pieces of skin obtained from the same animals ($n = 7$).

A useful simplification of the method of Aceves and Erlij (1971) was the elimination of the hydrostatic pressure step. We adopted this modification because we discovered that it was easy to remove the corium after the collagenase treatment by gently peeling it away from the epithelium, provided the outside surface of the skin had previously been glued to a Lucite ring (o.d., 3 cm; i.d., 2.6 cm) with a tissue adhesive (Zipbond, Tescom Corp., Minneapolis, Minn.), thereby maintaining the tissue as a flat sheet.

The techniques for microelectrode recording and estimation of electrical parameters are identical to those previously described in detail (Helman and Fisher, 1977*a*). Because the microelectrodes were advanced vertically from above, the surface to be penetrated was selected by mounting the epithelium with either the apical or basolateral surface upward. In the latter case, the epithelial cells and skin glands are directly exposed to the incubation solution (see Fig. 2 in Aceves and Erlij [1971] and Fig. 1 in Erlij [1971]). As described previously (Helman and Fisher, 1977*a*), E_1' is the transepithelial voltage when the voltage at the apical barrier is reduced to zero. R_o^f and R_i are the specific resistances of apical and basolateral membranes, and $\%R_o = R_o^f(R_o^f + R_i) \times 100$.

Statistical values are reported as means ± SE.

Epithelial K Analysis

To determine the potassium content, we removed the isolated epithelia from the chambers immediately after completion of the electrical measurements and blotted them gently on ashless filter paper to remove excess water. The epithelia were dried to constant weight on aluminum foil at 100°C. After complete digestion in concentrated HNO_3, and after appropriate dilution, the samples were analyzed for K in a flame photometer (Model 143, Instrumentation Laboratory, Inc., Lexington, Mass.). In the calculation of the intracellular K concentration, a dry wt:wet wt ratio of 0.2 and an extracellular space of 200 ml/kg wet wt (Aceves and Erlij, 1971) were assumed.

RESULTS

A summary of the electrical parameters obtained with microelectrode penetration through apical and basolateral membranes is shown in Table I and II. The control short-circuit currents were 30.2 ± 7.9 and 27.3 ± 8.6 $\mu A/cm^2$, respectively, and 10^{-5} M amiloride added to the apical bathing solution reduced these values to 1.1 ± 0.7 and 2.1 ± 0.8 $\mu A/cm^2$, respectively.

When the microelectrode was advanced through the basolateral border, penetration of a cell was signaled by an abrupt negativity that in most cases

remained stable. If the voltage was not stable, the microelectrode was advanced further into the epithelium until an abrupt change in negativity was again observed. This often could be done at least two or three times. In some cases, after a recording had been made from a stable cell, the microelectrode was advanced, and quite often a second or third cell could be impaled that gave essentially the same stable values that had been recorded from the first cell. It was also observed that, even with unstable punctures, the peak values

TABLE I

INTRACELLULAR ELECTRICAL PARAMETERS DETERMINED FROM THE APICAL SURFACE

Epithelium No.	V_o^{sc}		E_1'		$\%R_o$	
	Control	Amiloride	Control	Amiloride	Control	Amiloride
	mV		*mV*			
1	−70.7±1.66 (11)	−102.7±1.43 (18)	98.3±0.91	116.2±2.28	71.9±1.67	88.6±1.27
2	−62.3±2.76 (11)	−100.5±1.54 (8)	108.5±3.18	116.3±2.22	57.6±2.52	86.5±2.03
3	−72.9±2.05 (11)	−96.4±2.42 (9)	106.1±3.97	110.7±2.29	69.2±1.92	87.1±1.98
4	−49.2±1.55 (11)	−81.4±199 (13)	89.9±4.02	97.4±2.88	55.2±1.68	83.8±1.64
5	−90.9±3.08 (6)	−103.0±1.12 (8)	123.3±5.60	124.3±5.60	74.0±2.95	83.4±2.76
6	−76.1±2.86 (16)	−96.6±1.32 (11)	103.9±3.41	108.7±3.07	73.3±1.69	89.2±1.78
7	−95.5±2.47 (12)	−104.8±1.76 (9)	111.9±4.32	113.1±2.12	85.7±1.48	92.7±1.37
8	−45.9±2.80 (11)	−88.1±3.04 (5)	104.0±6.19	131.7±6.42	44.8±2.80	67.7±4.99
Mean±SEM	−70.4±6.77	−96.7±2.88	105.7±3.45	114.8±3.63	66.5±4.60	84.9±2.67

Number of cells measured is given in parentheses.

TABLE II

INTRACELLULAR ELECTRICAL PARAMETERS DETERMINED FROM THE BASOLATERAL SURFACE

Epithelium No.	V_o^{sc}		E_1'		$\%R_o$	
	Control	Amiloride	Control	Amiloride	Control	Amiloride
	mV		*mV*			
9	−89.9±1.08 (6)	−93.5±1.23 (4)	96.3±1.44	96.4±1.16	93.4±1.24	96.9±0.73
10		−95.8±1.67 (13)		103 (1)		100.4 (1)
11	−81.4±4.40 (7)	−114.4±1.42 (6)	107.7±2.78	116.5±3.50 (2)	75.4±2.40	100.2±0.2 (2)
12	−92.7±3.32 (5)	−98.7±1.82 (13)	104.1±2.50	95.5±3.31 (4)	89.0±1.86	100.3±1.35 (4)
13	−94.6±2.17 (7)	−101.7±1.32 (8)	104.8±2.14		90.3±1.18	
14	−99.3±1.70 (15)	−106.6±1.08 (14)	111.8±1.69	112.1±2.16 (14)	88.8±1.20	95.2±1.06 (14)
Mean±SEM	−91.6±2.97	−101.8±3.14	104.9±2.5	104.7±4.18	87.4±3.1	98.6±1.08

Number of cells measured is given in parentheses.

of intracellular negativity were essentialy the same as those recorded from stable cell penetration. In agreement with Nagel (1966*b*), who punctured from the apical side of the tissue, we consider the uniformity of intracellular voltages obtained with basolateral punctures to indicate a high degree of electrical coupling among the cells of the stratified epithelium.

The intracellular voltage, V_o^{sc}, recorded with microelectrodes in short-circuited epithelia, averaged −70.4 and −91.6 mV for apical and basolateral penetrations, respectively, for isolated epithelia obtained from two groups of frogs (Tables I and II). Although the values of V_o^{sc} obtained with apical punctures are somewhat less than those reported previously for intact skins

(Helman and Fisher, 1977*a*; Helman et al., 1979), they are similar to values observed in studies of intact skins from the same group of frogs ($V_o^{sc} = -84.4 \pm 4.7$ mV; $I_{sc} = 19.0 \pm 2.7$ μA/cm^2; $E_1' = 104.6 \pm 5.4$ mV).[1] As in previous studies with amiloride, inhibition of Na entry at the apical barrier of cells caused the V_o^{sc} to become more negative, approaching the magnitude of E_1' as the $\%R_o$ increased. In the present studies, the R_o^f averaged 5,017 ± 2,351 ($n = 8$) and 4,997 ± 1,068 ($n = 5$) Ω cm^2 for apical and basolateral routes of cell penetration, respectively. The R_i averaged 1,696 ± 376 and 583 ± 89 Ω cm^2 for apical and basolateral punctures, respectively. The values of R_i measured in intact skins obtained from many groups of frogs over several seasons have ranged from ~50 to >2,000 Ω cm^2, and so no special significance can be attributed to the differences in R_i observed here. It is of interest, however, that groups of skins of lowest R_i yield the higher mean V_o^{sc} values (Tables I and II).

Effects on V_o^{sc} of Increasing $[K]_i$

Fig. 1 shows a tracing of the changes in V_o^{sc} caused by increasing the

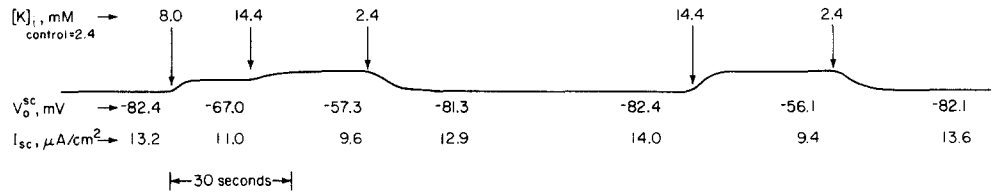

FIGURE 1. Changes in V_o^{sc} and I_{sc} upon elevation of the [K] of the basolateral (inner) solution. Note the rapid responses and the reversibility upon return to the control 2.4 mM $[K]_i$ solution.

potassium concentration in the inner (basolateral) solution. As shown in Fig. 1, for control epithelia (basolateral punctures), increasing the $[K]_i$ from 2.4 to 8.0 and then to 14.4 mM caused stepwise depolarizations of the V_o^{sc} that were accompanied by decreases in the I_{sc}. The changes in voltage were rapid, reaching new plateaus within ~5–10 s and were entirely reversible upon return to the control 2.4 mM $[K]_i$ solution. Similar results were obtained when amiloride pretreatment was used to inhibit the I_{sc}.

[1] It is not known why in some groups of skins the V_o^{sc} is lower and the R_i is higher than in other groups of skins. As amiloride at 10^{-5} does not cause $I_{sc} \rightarrow 0$, the $\%R_o$ would not be expected to be 100. Nevertheless, as we have observed, the $\%R_o$ can be significantly less than 100% at 10^{-5} M amiloride. This could be explained by imperfect sealing of the microelectrode, the existence of a non-amiloride-inhibitable conductance at the apical barrier, or relatively high R_i values. To the extent that the same criteria for acceptable cellular impalements apply to all groups of skins, we believe that inadequate sealing of the microelectrode is not the most likely explanation for the lower values of V_o^{sc}. Studies in our laboratory by Abramcheck and Helman (personal communication) have shown that relatively low concentrations of CO_2 (<5%) cause substantial increases in R_i, with concomitant decreases in V_o^{sc}. Thus, differences among groups of skins may, in part, be related to differences in metabolic rate, although other physiological processes certainly are possible factors.

When the data were normalized to reflect the change in voltage per 10-fold change in $[K]_i$, it became obvious that the relationship between V_o^{sc} and log $[K]_i$ was not strictly linear in the range of $[K]_i$ studied. In control epithelia, the ΔV_o^{sc} averaged 28.9 mV per decade when $[K]_i$ was changed from 8.0 to 14.4 mM. With amiloride outside, a similar pattern was observed, with the ΔV_o^{sc} being larger for changes in $[K]_i$ between 8.0 and 14.4 mM. When paired measurements were made in the same epithelium, the ΔV_o^{sc} per decade was larger for amiloride-treated tissues than for those under control, Na-transporting conditions. The mean of the paired ratio of amiloride to control responses of four isolated epithelia exposed to increases in the $[K]_i$ was 1.25 + 0.09 ($n = 8$). Under these circumstances, the basolateral barrier appeared to be highly sensitive to relatively small changes in the $[K]_i$, and such data are in accord with the suggestion of Koefoed-Johnsen and Ussing (1958) that the inner barrier of the skin is highly permeable to K.

K Content of Isolated Epithelia

Upon completion of a study with microelectrodes, the epithelium was removed from the chamber and analyzed for total K. K content ranged between 0.33 and 0.464 μeq/mg dry wt, averaging 0.392 ± 0.111 μeq/mg dry wt ($n = 16$). When these values were expressed as concentration in cell water, they averaged 122.5 ± 3.36 meq/liter cell water, with a range of 108.9–145. These values are similar to those reported by Aceves and Erlij (1971) and Ferreira (1979) for isolated epithelia, by Rick et al. (1978), who used electron microprobe analysis of intact skins, and by Zylber et al. (1973), who studied isolated cells of the skin.

DISCUSSION

Perhaps the most interesting point that emerges from this study is that the electrical parameters of the epithelium have essentially the same values, regardless of the route of penetration of the epithelium. The determinations also agree with those reported previously in intact skins punctured from the apical surface. The E'_1 values were somewhat lower, averaging nearly 105 mV, but they were not different from comparable studies performed concurrently with intact skins. Amiloride exerted its primary effect on the resistance of the apical barrier with little or no effect at the basolateral barrier. When the $[K]_i$ was elevated, the voltage at the inner barrier was depolarized in steps requiring, at most, 5–10 s, and the V_o^{sc} remained essentially constant for an additional 5–10-min period of observation. Thus, changes in V_o^{sc} caused by increases in $[K]_i$ were entirely reversible and reproducible from cell to cell in the same preparation. Similar studies with intact skins have indicated that ~10–15 min are required for increases in $[K]_i$ to depolarize the V_o^{sc} (Fisher and Helman, 1978; Fisher, 1979). This shows that the use of isolated epithelia makes possible observations of very rapid and direct effects at the basolateral barriers.

Our findings have particular bearing on the suggestion that negative voltages with reference to the apical solution of frog skin originate in the s. corneum (Engbaek and Hoshiko, 1957; Nunes and Lacaz Vieira, 1975).

Indeed, it has been suggested that such negative potentials arise from mechanical and chemical characteristics of the s. corneum (Nunes and Lacaz Vieira, 1975; Nelson et al., 1978). Regardless of the potentials associated with the s. corneum, the penetration through the basolateral surface rules out the possibility that our results represent artifacts caused by the specific properties of the s. corneum. This notion is supported by the finding that penetration of the cells of the s. corneum is not associated with a resistance barrier sensitive to procedures (amiloride, 0 $[Na]_o$) known to alter active transeptithelial Na transport (Nagel, 1976*a*; Helman and Fisher, 1977*a*; Helman et al., 1979). In fact, the resistance of the s. corneum is negligible. It contributes essentially nothing to the transcellular resistance of the epithelium (Helman and Fisher, 1977*b*).

In agreement with the findings with apical punctures, the intracellular voltages are similar regardless of the cell layer punctured. When the tissue is treated with amiloride or when the K concentration is elevated in the basolateral solution, the electrical behavior of the apical and basolateral barriers shows the expected asymmetrical responsiveness to these agents, regardless of the route of cell penetration. Moreover, because the intracellular voltage of all cell layers responds uniformly to changes in [Na], to amiloride in the apical solution, and to [K] in the basolateral solution, we suggest that the apical barrier corresponds to the apical face of the cells of the s. granulosum, whereas the basolateral barrier corresponds to the basolateral membranes of all cell layers that form the stratified epithelium.

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