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K^+-Permeability of the Outer Border of the Frog Skin (*R. temporaria*)

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Summary. Skins from *Rana temporaria,* investigated with microelectrode techniques in the absence of Na uptake across the outer border (Na-free epithelial solution or amiloride), were found to be permeable to K^+ at the apical membrane in 10–20% of the experiments. Full development of the K^+-permeable state requires the absence of Na^+ uptake for certain periods of time, which suggests that the K^+-permeability of the apical membrane is higher at lower intracellular [Na]. The addition of Ba^{++} reduces the K^+-permeability of the apical membrane. These skins may provide a model for the study of transcellular K^+ movements.

According to Koefoed-Johnson and Ussing [6], the electrophysiological effects of Na^+ entry across the outside-facing membrane of the frog skin can be explained by assuming that the apical membrane is essentially impermeable to K^+ and almost exclusively permeable to Na^+ when the bathing media are devoid of penetrant anions. This permeability pattern has been repeatedly confirmed [4, 8, 22]. In fact, the only condition under which a high K^+-permeability had been found is that which prevails after treatment of the apical membrane of the frog skin with polyene antibiotics such as amphotericin B [19] or Filipin [20] which were reported to induce the formation of K^+-permeable channels in these membranes. However, we have recently found that the apical border of skins from *R. temporaria* is often remarkably permeable to K^+ even before addition of a polyene antibiotic or any other agent [3]; similar observations have been reported by Zeiske and Van Driessche [23]. Therefore, the present investigation was undertaken to characterize the naturally-occurring K^+-permeability of the apical membrane of *R. temporaria* skin. This tissue might well provide a useful model for the study of transepithelial K^+-transport such as that found in some of the tight mammalian epithelia [1, 10]. It will be shown that the idea of an invariably low K^+-permeability of the apical membrane of *R. temporaria* skin is not warranted, that indeed a fairly high K^+-permeability is found in a significant minority of isolated, untreated skins.

Materials and Methods

The experiments were done on abdominal skins of *R. temporaria* using previously described microelectrode techniques [14–16]. The corial bathing solution was always a sodium-rich Ringer solution of the following composition (in mM): Na, 110; K, 2.5; Ca, 1.0; Cl, 112; HCO_3, 2.5; glucose, 10 (NaRi). The epithelial side was perfused with: NaRi alone or NaRi plus 10^{-4} M amiloride (NaRi-Am); another solution in which K^+ was substituted for all Na^+ (KRi), i.e., with KRi alone or KRi plus 10^{-4} M amiloride (KRi-Am); various mixtures of NaRi-Am plus KRi-Am; or with a Na-free choline-Ringer solution. The actual $[K^+]$ of each solution was measured by flame photometry (IL 543, Instrumentation Laboratories) after the experiment. The epithelial perfusion rate was comparatively low (5–10 ml/min) to permit stable positioning of the microelectrode within the cell for relatively long time periods.

The skins were short circuited except for periods when the transepithelial potential was voltage clamped to values of +20 mV for measurement of the fractional resistance at the outer border. The notations for each of the measurements obtained are the following: I_{sc} = short-circuit current; V_{sc} = intracellular potential of the short-circuited skin; $F(R_o)$ = fractional resistance at the outer border, which is operationally defined by the ratios $F(R_o) = R_o/(R_o + R_i) = \Delta V_o/\Delta V_t$, where ΔV_o and ΔV_t refer to the change in potential across the apical border and the entire epithelium, respectively, while R_o and R_i refer to the apical and basolateral membrane resistance, respectively.

The present study was complicated by the fact that skins from a small fraction only of a given batch of frogs from the same supplier were permeable to K^+ (*see* below). To find out whether this variation was related to different stages of the moulting cycle, the animals were kept in individual cages, half-immersed in tap-water, so that observations on the progress of moulting could be made. In these experiments the frogs were kept at 20–22 °C. Otherwise, the frogs were stored at 6–15 °C. The experiments were done between November and March.

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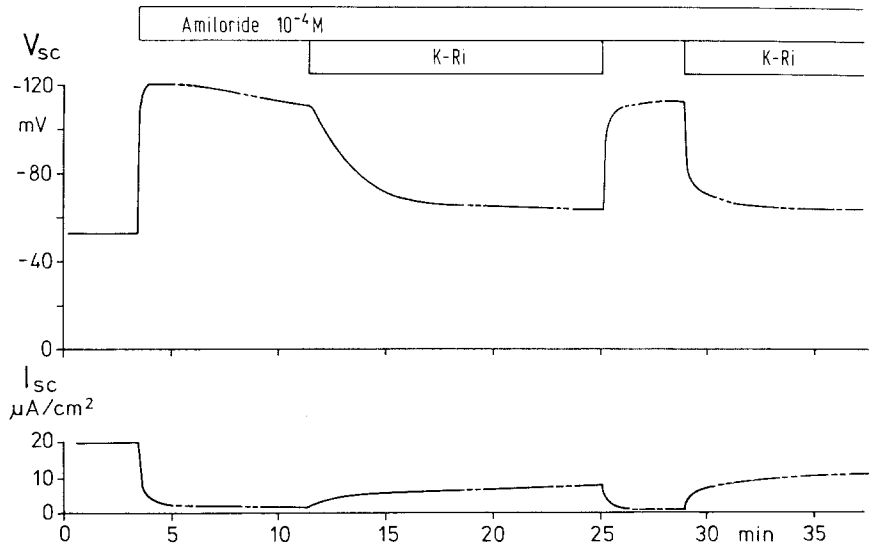

Fig. 1. Intracellular potential under short-circuit conditions (V_{sc}, upper trace) and short-circuit current (I_{sc}, lower trace) of frog skin epithelium after addition of amiloride (10^{-4} M) to the epithelial perfusion solution and subsequent substitution of Na^+ by K^+ (*K-Ri*). Note the instantaneous response of the V_{sc} upon removal and the second application of K-Ri

Results

Figure 1 shows the typical response of the intracellular potential (V_{sc}) and short-circuit current (I_{sc}) after substitution of K^+ for Na^+ in the epithelial perfusion solution (Ringer containing 10^{-4} M amiloride). The change from Na^+ to K^+ resulted in a decrease of the V_{sc} from -110 mV to -63 mV, and a concomitant increase of the I_{sc} by about 5 μA/cm² in the direction of positive ions flowing from the epithelial to the corial bathing fluid. Steady values of V_{sc} and I_{sc} were approached only after more than 5 min. Consequently, the observed changes of the V_{sc} might be due to a K^+-induced depolarization of the basolateral membranes resulting from penetration of the junctional complexes and entry of K^+ into the lateral intercellular spaces. However, the rapid reversibility of the K^+-induced changes argues strongly against this possibility: V_{sc} and I_{sc} returned immediately to control values after KRi-Am was removed from and NaRi-Am added back to the epithelial bathing compartment. In a consecutive substitution of K^+ for Na^+, the change of V_{sc} occurred instantaneously, demonstrating undelayed effectiveness of K^+ upon the apical border.

In other experiments, the response of V_{sc} and the I_{sc} to the application of KRi at the epithelial side was very rapid. Under conditions similar to those during the second change to KRi-Am in the above experiment (*see* Fig. 1), the entire K^+-induced drop of the V_{sc} was complete within less than 2 sec; the reverse change in epithelial perfusion (from K^+ to Na^+) occurred with similar velocity. These time courses are characteristic of those expected of K^+-induced diffusion potentials across the apical membrane.

Direct evidence for a K^+-selective conductance of the apical membrane was obtained from the linear dependence of V_{sc} on the fractional resistance of the outer border, $F(R_o)$, as observed after the application of KRi-Am (Fig. 2*a*). The statistically significant correlation between the K^+-induced changes in $F(R_o)$ and V_{sc} (i.e., between $\Delta F(R_o)$ and ΔV_{sc}) was calculated from the steady-state values during epithelial perfusion with NaRi-Am less the corresponding steady-state values during perfusion with KRi-Am. The decrease in V_{sc} must result from a decrease of the apical membrane resistance. This claim was substantiated by the demonstration that ΔV_{sc} was a near-linear function of ΔI_{sc} (Fig. 2*b*). Thus, the decrease of $F(R_o)$, together with the increase of the transcellular current, implies that R_o *per se* is primarily decreased.

Figure 3 illustrates that the degree of change in V_{sc} depends upon the epithelial $[K^+]$. After reaching a control value of V_{sc} of -95 mV during perfusion of the epithelial side with NaRi-Am (containing 2.5 mM K^+), the value of V_{sc} decreased in consecutive steps to -86, -75, and -61 mV following perfusion of the epithelial side with 10, 30, and 112 mM KRi-Am, respectively. During the intermittent periods of perfusion with the control solution of NaRi-Am, V_{sc} always returned to the control level. Not shown are the increases of the I_{sc} that occurred concomitantly with the decreases of the V_{sc}.

The results obtained from 5 frogs are summarized in the form of semilogarithmic plots of the values of V_{sc} against those of epithelial $[K^+]$ (Fig. 4*a*) and the values of V_{sc} against those of I_{sc} (Fig. 4*b*). The plot of each set of values from individual cells (connected by lines) generated a fairly linear set of functioned correlations. The average slopes, calculated by linear regression analysis of all values, are 29.6 mV/

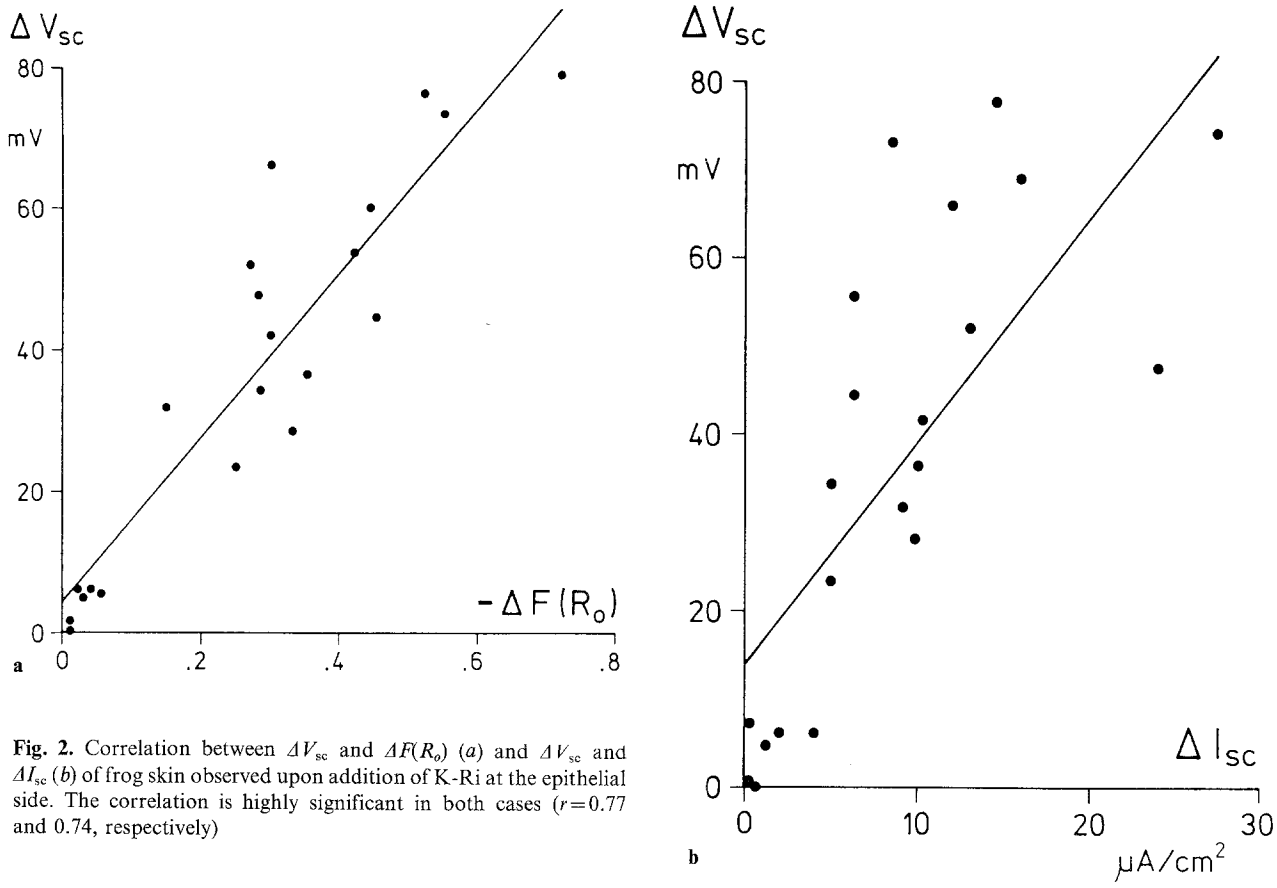

Fig. 2. Correlation between ΔV_{sc} and $\Delta F(R_o)$ (*a*) and ΔV_{sc} and ΔI_{sc} (*b*) of frog skin observed upon addition of K-Ri at the epithelial side. The correlation is highly significant in both cases ($r=0.77$ and 0.74, respectively)

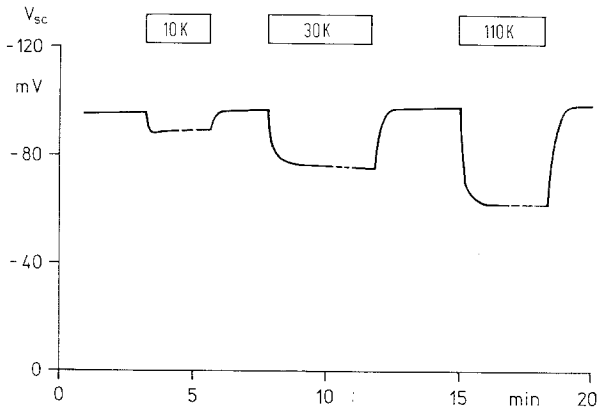

Fig. 3. Response of V_{sc} to increasing $[K^+]$ of the epithelial perfusion solution. All solutions contained 10^{-4} M amiloride; $[K^+]$ during control periods was 2.5 mM

tenfold change of $[K^+]$ and 3.6 mV/μA · cm^{-2}, respectively.

The observations encompassed in this study were obtained from a comparatively small fraction of all skins investigated during the same period. Only 10 to 20% of the skins from *R. temporaria*[1] were permeable to K^+. Furthermore, the degree of permeability was rather variable. Attempts to correlate the existence or the degree of K^+-permeability to other functional properties were not successful. Selection of frogs during certain stages of the moulting cycle (immediately after shedding of the str. corneum until 14 days after observed moulting) did not reveal detectable differences in K^+-permeability. Similarly, the storage of the frogs in warm or cold environment had no systematic influence upon the permeability characteristics. Finally, the varying degree of K^+-permeability during the winter and spring (in the present group of frogs) was the same as that found during the summer (in another group), thus ruling out the dependence upon seasonal influences.

The permeability of the apical membrane to K^+ is not dependent upon the presence of amiloride. V_{sc}, I_{sc} and $F(R_o)$ in the presence of epithelial K^+ remained essentially constant whether the KRi-solution contained amiloride or not. Increasing the amiloride concentration to 10^{-3} M had no effect upon the permeability properties of the outer border. On the other hand, the absence of Na uptake across the apical border is required for the elicitings of any detectable K^+-permeability. Even with as little as 1–3 mM Na^+

[1] Repeated tests on skins of *R. esculenta*, *Bufo bufo* and *Bufo marinus* and on bladders of *B. marinus* provided no indication of significant K^+ influx through the apical cell membrane.

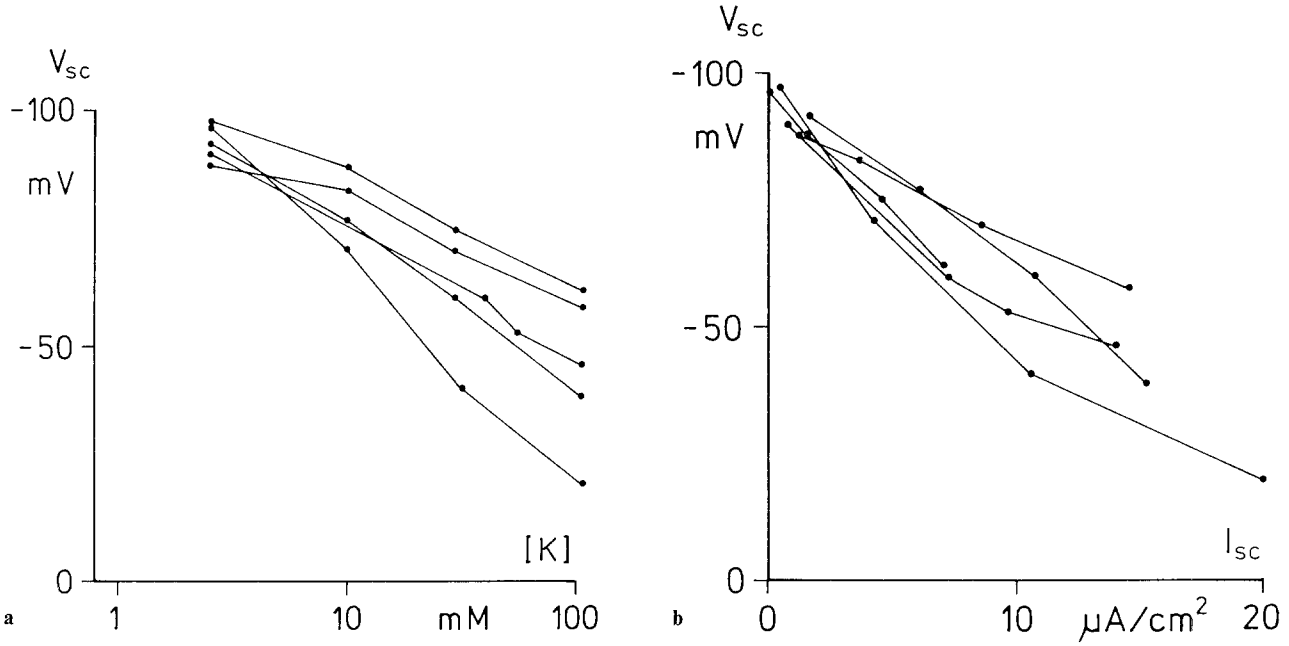

Fig. 4. Relationship between V_{sc} and $[K^+]$ of the epithelial perfusion solution on a semilogarithmic scale (*a*) and I_{sc} during epithelial application of the different [K–] (*b*). Points, connected by lines, indicate measurements from the same cell of one frog skin (5 different experiments)

in the epithelial solution, we were unable to detect any finite K^+-permeability.

The full development of the K^+-permeable state in K^+-permeable skins apparently requires a certain period of exposure of the epithelial side to K^+. This is illustrated by an experiment in which the epithelial perfusion solution was changed directly from NaRi to KRi (Fig. 5). As expected from the assumption that K^+ would not penetrate the outer membrane, V_{sc} increased instantaneously to values of around -100 mV and the $F(R_o)$ to a value of 0.93. Within the following 3–4 min, V_{sc} and $F(R_o)$ decreased to values of -50 mV and 0.60, respectively. It was therefore inferred that the apical membrane becomes increasingly permeable to K^+ during these 3 to 4 min. Microelectrode unsealing, which would also explain the result, is unlikely because the control values were again approached upon perfusion with NaRi and because subsequent perfusion with choline Ringer resulted in changes of V_{sc} and $F(R_o)$ which are typical of intracellular location of the microelectrode.

The effect of Ba^{++}, a known inhibitor of passive transmembranal K^+ fluxes in skeletal muscle [12], was to cause a rapid inhibition of the K^+-induced permeability of the apical membrane (Fig. 6), as deduced from the fact that a K^+-induced depolarization of the V_{sc} (to a steady value of -56 mV) was partially restored to a near control value of -78 mV upon addition of 0.5 mM Ba^{++} to the KRi-Am solution. Simultaneously, the I_{sc} decreased and the $F(R_o)$ increased to values indicating drastically reduced permeability of the apical border. The changes induced by Ba^{++} as well as by K^+ were completely reversible upon withdrawal of Ba^{++} and K^+, respectively. The rapidity of onset and disappearance of these effects again supports the view that the observed events occurred at the apical border.

Discussion

Evidence that K^+ can penetrate the outer border of the frog skin has previously been reported by others. Huf and Wills [5] reported that K^+ is ejected into the epithelial bathing solution at transepithelial potentials above 30–40 mV (corial side positive). Similarly, Levi and Ussing [7] found that K^+ fluxes deviate from simple passive behavior and suggested that part of this flux occurs by way of a transcellular pathway. Data on the uptake of K^+ compared to that of the extracellular marker (PEG [12, 13]) across the outer border also suggest that this border is permeable to K^+. On the other hand, extensive electrophysiological studies provide evidence of a low but nonetheless finite permeability of the outer border of amphibian skins and bladders to K^+ [8, 4]. The discrepancy between the present findings and those of others [8, 4] could be ascribed to species differ-

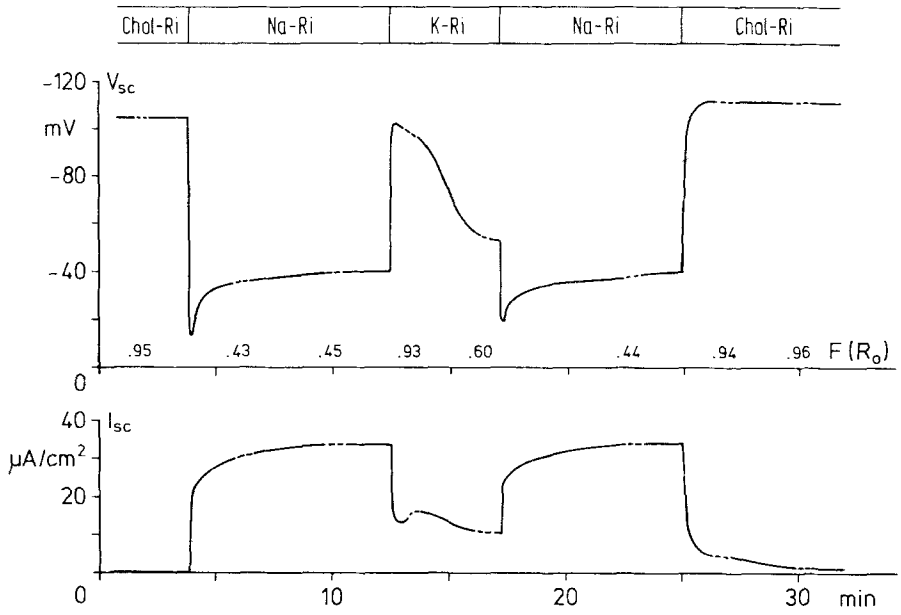

Fig. 5. Response of V_{sc} (upper trace) and I_{sc} (lower trace) upon change of the epithelial perfusion solution from Na-Ri to K-Ri or choline-Ringer. Note the instantaneous increase of the V_{sc} and the $F(R_o)$ to almost identical values during both periods of Na-free perfusion

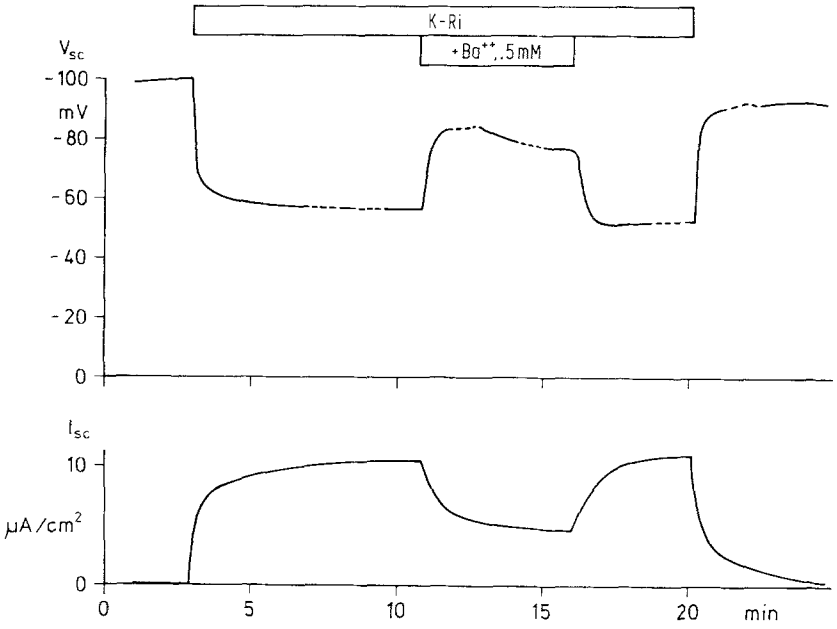

Fig. 6. Effect of Ba^{++} (0.5 mM, epithelial side) upon the depolarization induced by K-Ri at the epithelial side. All solutions contained 10^{-4} M amiloride

ences. In the present investigation, permeability of the apical membrane to K^+ was observed only in skins of *R. temporaria*, and similar observations have been made by others [23]. The vanishingly low K^+-permeability of *R. temporaria* skins studied by Koefoed-Johnsen and Ussing [6] indicates that the apical membrane even of this species is not invariably permeable to K^+. In fact, most of the skins in our present study were K^+ impermeable. At present the factors which determine the degree of K^+-permeability of the skin are unknown. Seasonal variations, environmental temperature during storage of the animals, and stage during the natural moulting cycle have been ruled out as decisive conditions. Hormonal, metabolic, and dietary factors remain to be investigated in subsequent studies.

The present data suggest that the apical cell membrane is the site of K^+ fluxes across the outer border of the epithelium. The possibility that the observed effects of high $[K^+]$ upon the intracellular potential result from penetration of K^+ through paracellular shunt pathways (generating the change of I_{sc}) and

depolarization of the basolateral membrane can be excluded because of (i) the rapid onset, the reversibility, and the magnitude of the changes in V_{sc}, (and I_{sc}) upon addition of K^+ at the epithelial side and (ii) the concomitant change in the fractional resistance of the apical membrane, $F(R_o)$. The latter observation requires a decrease in the resistance of the apical cell membrane in order to be consistent with the observed increase in I_{sc}. Although ion fluxes through the paracellular shunt may contribute to part of the transepithelial current, the effect of such fluxes on V_{sc} appears to be small[2].

Barium-induced blockage of passive K^+ movements have been found previously in membranes of cardiac muscle [2] and gastric mucosa [21]. Recently, a similar effect of Ba^{++} on basolateral membranes of the frog skin has been reported [17]. The fact that Ba^{++} is also effective in blocking K^+ fluxes across the apical membrane of the frog skin (Fig. 6) suggests that the K^+ channels in the apical membrane of the frog skin cells possess some of the characteristics of other biological K^+ channels. Accordingly, skins with naturally occurring K^+-permeability may provide useful insights into the mode of permeation across epithelial membranes.

Although the list of factors which determine the K^+-permeability of cell membranes is as yet incomplete, two factors can be implicated as determinants of the K^+-permeability in *R. temporaria* skins, namely, the concentration of K^+ in the epithelial fluid and that of Na^+ in the cellular fluid. Increase of K^+-permeability with increasing duration of exposure of the epithelial side to high $[K^+]$ has been reported by Zeiske and Van Driessche [23] and confirmed in the present study. Whether this effect coincides with a loss of specificity [23] cannot be revealed by the present technique. Evidence that the intracellular $[Na^+]$ modulates the K^+-permeability of the apical membrane is provided by data from the experiments shown in Figs. 1 and 5. The K^+-permeable state was approached slowly within about 8 min of blocked Na^+ entry across the apical membrane (Fig. 1, first period of KRi-Am). During this period, V_{sc} was hyperpolarized to values which exceed those of the K^+ equilibrium potential. It has been suggested that this hyperpolarization is the result of rheogenic Na^+ transport across the basolateral membrane and indicates presence of Na^+ in the cytoplasmic tissue compartment [18]. Further pump-induced reduction of the cellular $[Na^+]$ with increasing time of exposure to amiloride results in a partial disappearance of the initial hyperpolarization (reduced intracellular $[Na^+]$). Under these conditions the substitution of K^+ for Na^+ in the epithelial perfusion solution results in instantaneous response of the V_{sc}. Similar observations were obtained in other experiments, indicating that the final steady-state level of K^+-permeability was approached more rapidly if the application of K^+ was preceded by longer periods of absent Na^+ influx into the cell and presumably lower cellular $[Na^+]$. Such an inverse relationship between $[Na^+]$ and K^+-permeability would also explain the result of the experiment shown in Fig. 5.

Permeability of the apical border to K^+ cannot be detected in the presence of Na^+-uptake – even from a solution with $[Na^+]$ as low as 1–3 mM. This might be the result of technical limitations, i.e., the near-impossibility of detecting small increments of K^+ current in the presence of comparatively high Na^+ currents. On the contrary, the change from choline to K^+ in the presence of 1–3 mM Na is always followed by a *reduction* of the I_{sc} together with a change to a more negative V_{sc} (*unpublished results*). This result is in accord with that of Mandel and Curran [11] who showed that K^+ at the epithelial side inhibits Na^+-permeability and supports the notion that a depletion of the intracellular Na^+ is necessary for the development of a significant level of K^+-permeability of the apical border of *R. temporaria*. Further studies, including direct correlation with measured intracellular Na^+ activities, are warranted to verify this presently speculative property of the apical membrane in an epithelium which is permeable to Na^+ and K^+, and which thereby provides a useful model for the study of transcellular movement of these two ions.

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[2] Skins, initially impermeable to K^+, were made highly permeable to K^+ across the paracellular shunt pathway by the technique of adding hypertonic urea to the epithelial side [22, 9]. Such urea-treated skins did not show significant changes in V_{sc} and $F(R_o)$ upon addition of K-Ringer to the epithelial side, even in the presence of transepithelial K^+ fluxes presumably through the K^+-permeable paracellular path (*unpublished results*).

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