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Basolateral Membrane Potential and Conductance in Frog Skin Exposed to High Serosal Potassium

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Summary. In studies of apical membrane current-voltage relationships, in order to avoid laborious intracellular microelectrode techniques, tight epithelia are commonly exposed to high serosal K concentrations. This approach depends on the assumptions that high serosal K reduces the basolateral membrane resistance and potential to insignificantly low levels, so that transepithelial values can be attributed to the apical membrane. We have here examined the validity of these assumptions in frog skins (*Rana pipiens pipiens*). The skins were equilibrated in NaCl Ringer's solutions, with transepithelial voltage V_t clamped (except for brief perturbations ΔV_t) at zero. The skins were impaled from the outer surface with 1.5 M KCl-filled microelectrodes ($R_{el} > 30$ MΩ). The transepithelial (short-circuit) current I_t and conductance $g_t = -\Delta I_t/\Delta V_t$, the outer membrane voltage V_o (apical reference) and voltage-divider ratio ($F_o = \Delta V_o/\Delta V_t$), and the microelectrode resistance R_{el} were recorded continuously. Intermittent brief apical exposure to 20 μM amiloride permitted estimation of cellular (*c*) and paracellular (*p*) currents and conductances. The basolateral (inner) membrane conductance was estimated by two independent means: either from values of g_t and F_o before and after amiloride or as the ratio of changes ($-\Delta I_c/\Delta V_i$) induced by amiloride. On serosal substitution of Na by K, within about 10 min, I_c declined and g_t increased markedly, mainly as a consequence of increase in g_p. The basolateral membrane voltage V_i ($= -V_o$) was depolarized from 75 ± 4 to 2 ± 1 mV [mean ± SEM ($n = 6$)], and was partially repolarized following amiloride to 5 ± 2 mV. The basolateral conductance increased in high serosal K, as estimated by both methods. Essentially complete depolarization of the basolateral membrane and increase in its conductance in response to high [K] were obtained also when the main serosal anion was SO_4 or NO_3 instead of Cl. On clamping V_t over the range 0 to +125 mV in K_2SO_4-depolarized skins, the quasi-steady-state V_o/V_t relationship was linear, with a mean slope of 0.88 ± 0.03. The above results demonstrate that, in a variety of conditions, exposure to high serosal K results in essentially complete depolarization of the basolateral membrane and a large increase in its conductance.

Key Words frog skin · membrane potential · voltage clamp · K^+ depolarization

Introduction

In recent years, in order to characterize factors modulating the entry of sodium into anuran epithelia, much attention has been directed to outer (apical) membrane current-voltage relationships. In an attempt to avoid the substantial difficulties of intracellular electrophysiologic measurements, Fuchs et al. (1977) have suggested the use of serosal bathing solutions of high K concentration, reasoning that these should largely eliminate the resistance and electrical potential gradient across the basolateral membrane assumed to be permselective to K^+ (Koefoed-Johnson & Ussing, 1958). Using microelectrode techniques to evaluate the effects of high serosal [K], several investigators have reported that a significant basolateral membrane potential and resistance persist under these conditions (for example Nagel, 1977; Benos et al., 1983; Tang & Helman, 1983; DeLong & Civan, 1984). However, no comprehensive investigation of these issues has been reported. In the present study we found that in frog skins that were equilibrated in isosmotic NaCl, Na_2SO_4, or $NaNO_3$ Ringer's solutions, the basolateral depolarization induced by the corresponding K salt in the serosal (inner) solution is practically complete, and membrane resistance is reduced. These observations partially validate the use of the high K techniques introduced by Fuchs et al. (1977), at least under steady-state conditions. Some of these results have been presented previously (Klemperer et al., 1984).

Materials and Methods

Sections of abdominal skins of *Rana pipiens pipiens* (Connecticut Valley Biological or KONS, Germantown, Wisconsin) were mounted horizontally (mucosa up) in an Ussing type chamber (0.36 cm^2), as described by Nagel (1978). Tissues were perfused with NaCl Ringer's solutions (NaCl R) containing 110 mM NaCl, 1 mM $CaCl_2$, and 2.5 mM KOH buffered with 3.5 mM HEPES, pH 7.8, or where indicated, NaCl was replaced by either 55 mM Na_2SO_4 or 110 mM $NaNO_3$. Elevated serosal [K] was obtained by completely replacing Na by K. The osmolality of the solutions (measured by a Precision System osmometer) was 230 mOsm/kg

H_2O (adjusted by addition of mannitol in the case of SO_4 R). The transepithelial potential V_t was measured by calomel electrodes via saturated KCl-agar bridges equipped with continuously flowing 3 M KCl solution. An automatic voltage-clamp system (*see* Nagel et al., 1983) enabled us to clamp V_t by feeding current through two circular Ag/AgCl electrodes, and record the transepithelial current I_t and the total conductance g_t continuously. The latter was obtained from the deflection in current induced by a 10-mV transepithelial pulse for 0.3 sec at 1 Hz frequency. Exposure to 20 μM amiloride for about a minute then permitted estimation of the amiloride-insensitive conductance g_p and, by difference, the amiloride-sensitive conductance g_c[1]. The repetitive triggering by the voltage clamp allowed also continuous measurement of the voltage divider ratio of the outer membrane ($F_o = \Delta V_o/\Delta V_t$). Micropipettes were pulled into 9-mm tip length from self-filling borosilicate tubing (Kwikfil 1B 120F, WP-Instruments), using a horizontal puller (Industrial Science Assoc., Model 1). Microelectrodes, filled with 1.5 M KCl, had a resistance higher than 30 MΩ when measured in Ringer's solutions. The apical membrane potential V_o, referred to the mucosal solution, was measured via an Ag/AgCl wire connected to an electrode preamplifier. The microelectrode input resistance R_{el} was monitored by sending constant current pulses through the microelectrode periodically, as described by Nagel et al. (1983). All parameters were recorded simultaneously on chart records (Hewlett Packard Model 7100B) and occasionally on a Digital LSI 11-23 computer equipped with an analog-to-digital conversion board (Data Translation Model 2762). In addition, V_o was displayed on an oscilloscope (Tektronix, Inc. Model 516A).

Solutions were changed by use of a magnetic valve (mucosa) (Angar Scientific) or a noninterrupting manual valve (serosa). This allowed continuous measurements even during solution change, without loss of the microelectrode impalement. Impalement of the skin was performed from the mucosal side, with the electrode mounted on a stepping motor micromanipulator (E. Nagel, Biomedizinische Instrument, Munich, FRG) perpendicular to the skin surface. The electrode was advanced into the tissue in 3-μm steps. Upon cell penetration an instantaneous change in the potential was recorded, as well as a rise in F_o and R_{el}. The electrode was then withdrawn in 0.4-μm steps until R_{el} returned to a value equal to or less than before impalement (*see* Results). Strict criteria for the acceptability of an impalement were as previously described (Nagel, 1976, 1978; Garcia-Diaz et al., 1985). Results are expressed as mean ± SEM.

List of Symbols

- F_o voltage divider ratio across the outer (apical) membrane
- g_c transcellular (amiloride-sensitive) conductance
- g_i inner (basolateral) membrane conductance calculated according to Eq. (1)
- G_i inner (basolateral) membrane conductance calculated according to Eq. (2)
- g_p passive (amiloride-insensitive) conductance
- g_t transepithelial conductance
- I_c cellular (amiloride-inhibitable) current
- I_p paracellular (amiloride-insensitive) current
- I_t transepithelial current (usually at short circuit)
- R_{el} microelectrode resistance
- V_o outer (apical) membrane potential; cell with respect to the mucosal solution
- V_t transepithelial potential; serosal solution with respect to the mucosal solution

[1] In several skins, exposure to amiloride for about one minute did not produce complete effects on I_t and g_t. Longer exposures were avoided in order to shorten the recovery following removal of amiloride, so as to minimize the likelihood of dislodgement of the microelectrode and permit observations on the same cell before and after substitution of K for Na. Thus, our value of amiloride-sensitive current and conductance must be considered lower bounds of the cellular current and conductance.

Results

Microelectrode Artifacts

As has been emphasized previously (Garcia-Diaz et al., 1985), continuous monitoring of the microelectrode resistance during the impalement is of critical importance for the accurate measurement of membrane potential, especially when the value of V_o is small. The experiment depicted in Fig. 1 clearly demonstrates this necessity. In this particular experiment the skin was equilibrated in isotonic Na_2SO_4 R solution at the serosal surface and $NaNO_3$ R at the mucosal surface. The skin was "depolarized" by use of isotonic serosal K_2SO_4 R solution, and after 10 min V_o reached a value of 0 mV (*not shown*), which on the basis of our several criteria was considered reliable. At this time the electrode was taken out of the cell, and another impalement was obtained with the same electrode, as described in the figure legend. In period 1 a measurement of V_o gave a value of −15 mV, which would have seemed reliable if judged solely by a very stable reading of V_o and F_o, with the resistance of the electrode being only 4 MΩ higher than its initial value. However, knowing that $V_o = 0$ mV from the previous measurement encouraged us to withdraw the electrode slightly. Upon a withdrawal of 1 μm, V_o rose from −15 mV to 0 mV, with a concomitant drop in R_{el} (period 2). F_o remained unchanged, indicating that during withdrawal we had not crossed a significant resistance barrier or introduced a leak around the microelectrode. Application of amiloride verified the validity of the impalement and, furthermore, taking the electrode out of the tissue induced no change in R_{el}, indicating that no artifact was involved in measurement of V_o during period 2. Hence, it appears that upon crossing the stratum corneum prior to period 1, the electrode was slightly broken, as judged by the values of R_{el} before and after the impalement. In almost all impalements of frog skin we find situations where partial withdrawal of the microelectrode is necessary to reduce R_{el} close to the initial levels (*see also* Garcia-Diaz et al., 1985). The associated changes in

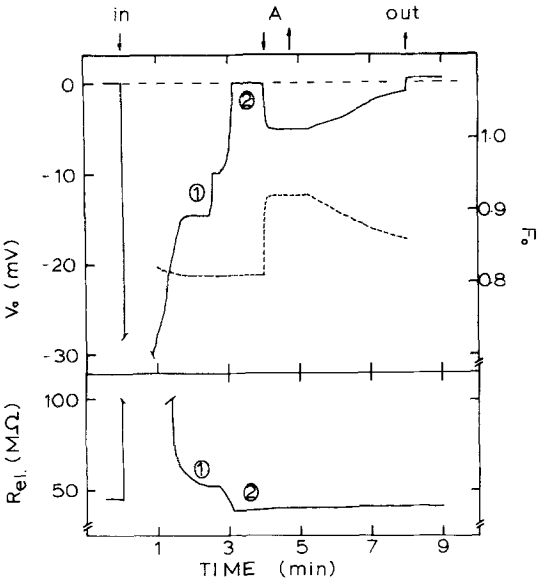

Fig. 1. Possible explanation for erroneous intracellular potential measurement. A skin exposed to mucosal $NaNO_3$ R was depolarized with serosal K_2SO_4 R. After reaching a steady state (with V_o = 0 mV), the electrode was withdrawn from the cell (R_{el} = 48 MΩ) and at the time marked by the arrow "in," the microelectrode was advanced into the tissue, as described under Materials and Methods. Upon touching the surface there was a jump in V_o, probably due to bending of the electrode tip; F_o was still zero and R_{el} was off scale. Impalement of the cell is indicated by the sharp drop in V_o to −30 mV, as well as an instantaneous rise in F_o (dashed line). At this time the electrode was withdrawn from the cell in 0.4-μm steps in order to reduce R_{el}. At the point indicated by "1," a stable recording of V_o = −15 mV was obtained and R_{el} was 52 MΩ. However, further withdrawal of the electrode from the cell brought another drop in V_o, to 0 mV, with a concomitant reduction of R_{el} to 45 MΩ, indicated by "2." (Note that there was no change in F_o.) Amiloride was applied for 1 min (↓ A ↑), and at 8 min (↑ out) the electrode was removed from the tissue. The offset in electrode potential was then 1 mV and R_{el} was 45 MΩ

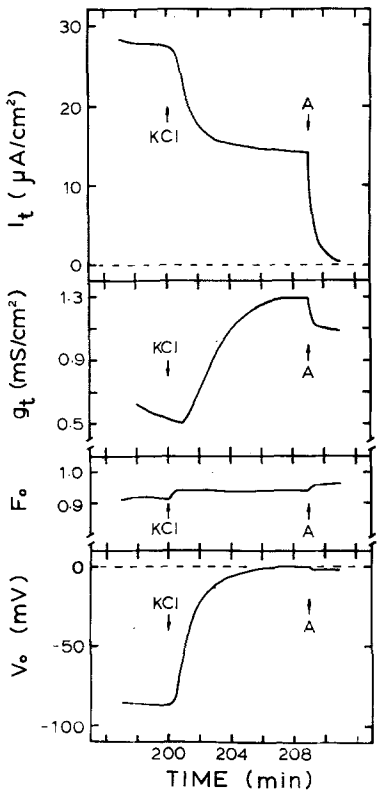

Fig. 2. Effects of serosal K (Cl Ringer's solution). The short-circuited skin was equilibrated in NaCl R solution bilaterally, as indicated under Materials and Methods. Serosal NaCl R solution was then replaced by KCl R solution, as indicated by the arrow, and the time course of effects on I_t, g_t, F_o, and V_o was observed. After achievement of a steady state, amiloride (2×10^{-5} M) was applied to the mucosal surface (arrow A). (Current flow I_t is taken as positive from mucosa to serosa; the cell potential V_o is referred to the mucosal solution.)

V_o however are highly variable (from almost no change up to 20 mV). Sometimes we were not able to reduce R_{el} by withdrawing the electrode and eventually the impalement was lost.

Effects of Serosal K (Cl-Ringer's Solutions)

The time course of representative effects of replacing serosal Na by K, in a frog skin pre-equilibrated bilaterally in NaCl R solutions is depicted in Fig. 2. As was observed by other investigators (e.g. Varanda & Vieira, 1978; Rick et al., 1984), substitution of K for Na causes a drop in I_t. After a 1-min period g_t started to increase, reaching a steady-state value within 10 min. V_o was depolarized in association with a slight increase in F_o. (The latter is usually not observed; *see* Table 1). Since similar results were obtained with HEPES or HCO_3 (2.5 mM) buffer, the combined data are summarized in Table 1.

Table 1 shows that the decrease in the steady-state level of I_t resulted largely from the 50% decrease of the amiloride-sensitive cellular current, whereas the very large increase in g_t is due to a 260% rise in the passive (amiloride-insensitive) conductance.[1] The steady-state level of V_o following equilibration was −2 ± 1 mV. The time required for the depolarization (about 10 min) agrees well with that reported by Nagel (1980).

The basolateral membrane conductance can be calculated by two different methods. The first makes use of the amiloride-sensitive conductance g_c and the voltage divider ratio F_o. If after 1 min of amiloride application the apical ion flux is not completely abolished, the influence of residual apical

Table 1. Effects of serosal K (Cl Ringer's solution)[a]

	Serosal/Mucosal	
	NaCl/NaCl	KCl/NaCl
I_t (μA/cm^2)	34.2 ± 2.7	11.6 ± 2.7
I_c (μA/cm^2)	26.2 ± 2.7	11.7 ± 1.4
g_t (mS/cm^2)	1.01 ± 0.21	2.87 ± 0.55
g_p (mS/cm^2)	0.71 ± 0.20	2.54 ± 0.47
g_c (mS/cm^2)	0.30 ± 0.04	0.34 ± 0.12
g_i (mS/cm^2)	2.2 ± 0.5	4.7 ± 1.0
G_i (mS/cm^2)	0.74 ± 0.1	6.2 ± 1.8
V_o (mV)	−75 ± 4	−2 ± 1
V_o (mV) [Amil]	−111 ± 2	−5 ± 2
F_o	0.79 ± 0.03	0.78 ± 0.03
F_o [Amil]	0.95 ± 0.01	0.86 ± 0.04

[a] Frog skins were equilibrated bilaterally in NaCl Ringer's solution buffered with either HEPES (n = 3) or HCO_3 (n = 3). Amiloride was applied for 1 min and then washed out. Upon restoration of all electrophysiological parameters (20 to 30 min), serosal Na was replaced by K. After a steady state was achieved (10 min), amiloride was applied again for 1 min. I_c (cellular current) is evaluated as the amiloride-sensitive current, and g_c (cellular conductance) is the amiloride-sensitive conductance.[1] g_i and G_i (basolateral conductance) were calculated as explained in the text.

conductance is compensated for by use of an equation following the treatment of Frömter and Gebler (1977) (Method I):

$$g_i = g_c/(F_o^A - F_o). \quad (1)$$

Here the superscript A indicates values in the presence of amiloride. The second method employs the changes in quasi-steady-state values of I_t and V_i produced by amiloride at short circuit:

$$G_i = -\Delta I_c/\Delta V_i. \quad (2)$$

As discussed in detail elsewhere (Nagel, 1985) (*see also* Discussion) the calculations usually do not agree, for reasons that are not clear. Under the experimental conditions of the present study, g_i and G_i changed in the same direction. For the experiments shown in Table 1, g_i increased from 2.2 ± 0.5 to 4.7 ± 1.0 mS/cm^2 and G_i from 0.7 ± 0.1 to 6.2 ± 1.8 mS/cm^2.

Effects of Serosal K (SO_4 Ringer's Solution)

In an attempt to minimize cell swelling, various investigators have employed K sulfate solutions, both with and without supplementary mannitol, in order to depolarize the inner membrane potential (e.g. Fuchs et al., 1977). This might seem *a priori* not the best choice, since the activity coefficient of K is only 0.43 in K_2SO_4 solutions of near physiological ionic strength (Robinson & Stokes, 1968). This suggests that only a partial effect might be expected, as compared to the effects of KCl. However, application of isotonic K_2SO_4 Ringer's to the serosal side of a tissue previously equilibrated bilaterally for 40 to 60 min in Na_2SO_4 Ringer's resulted in complete depolarization after 10 min (Fig. 3). g_t rose, in this case with increase of F_o, as well as decrease in the cellular current (*not shown*). Table 2 summarizes the results of three experiments. The depolarization was partially reversed by bathing the skins in serosal Na_2SO_4 solutions, i.e., V_o repolarized to −21 ± 4 (n = 3), and F_o fell to its original level (0.62 ± 0.07). As was the case in Cl-Ringer's solutions, ba-

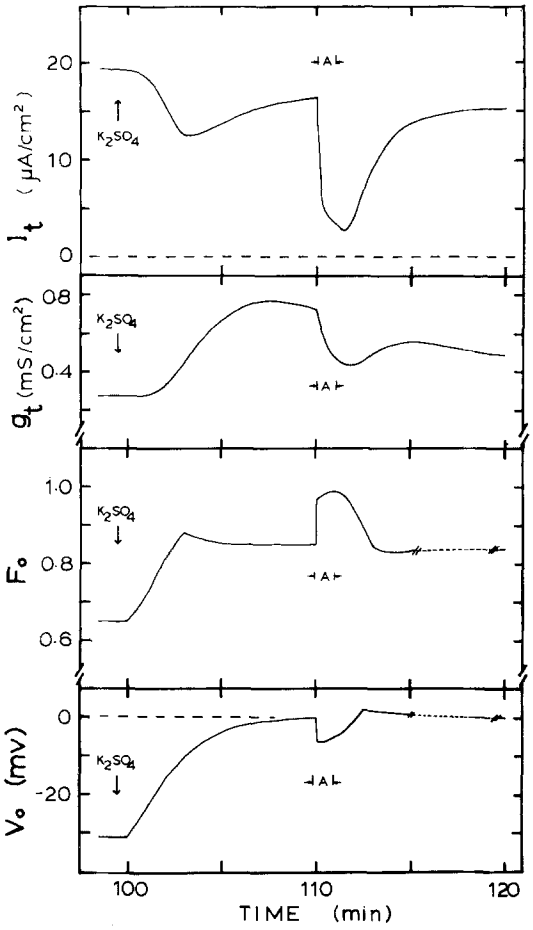

Fig. 3. Effects of serosal K (SO_4 Ringer's solution). The short-circuited skin was equilibrated in Na_2SO_4 R solution bilaterally. Na_2SO_4 R was replaced by K_2SO_4 R at the time indicated by the arrow. After achievement of a steady state, 2 × 10^{-5} M amiloride was applied to the mucosal surface for one min (→ A ←). The intracellular recordings from 100 to 115 min are from a single impalement. At that time the electrode was taken out of the cell and at 120 min another impalement was obtained using the same electrode

Table 2. Effects of serosal K (SO_4 Ringer's solution with mannitol)[a]

	Serosal/Mucosal	
	Na_2SO_4/Na_2SO_4	K_2SO_4/Na_2SO_4
I_t ($\mu A/cm^2$)	23.2 ± 0.6	15.9 ± 2.8
I_c ($\mu A/cm^2$)	15.2 ± 1.0	10.6 ± 2.2
g_t (mS/cm^2)	0.40 ± 0.12	0.69 ± 0.04
g_p (mS/cm^2)	0.24 ± 0.13	0.50 ± 0.03
g_c (mS/cm^2)	0.16 ± 0.01	0.19 ± 0.05
g_i (mS/cm^2)	0.48 ± 0.01	1.17 ± 0.35
G_i (mS/cm^2)	0.27 ± 0.03	2.1 ± 0.8
V_o (mV)	−32 ± 3	1 ± 2
V_o (mV) [Amil]	−90 ± 9	−4 ± 2
F_o	0.64 ± 0.01	0.79 ± 0.02
F_o [Amil]	0.97 ± 0.01	0.96 ± 0.02

[a] Skins were treated as in Table 1, except for the use of SO_4 Ringer's solutions (n = 3).

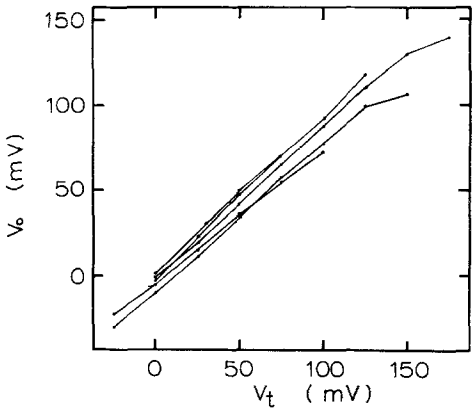

Fig. 4. V_o *vs.* V_t in K_2SO_4-depolarized skins. Skins were bathed for more than 20 min in K_2SO_4 R (serosa) and $NaNO_3$ R (mucosa). The transepithelial potential was clamped to the indicated values for 20 sec, followed by a 20-sec period at short circuit. The slope for V_t = −25 to 125 mV is 0.88 ± 0.03 (n = 5)

solateral conductance increased in high serosal [K]: g_i from 0.48 ± 0.01 to 1.2 ± 0.4 mS/cm^2 and G_i from 0.27 ± 0.03 to 2.1 ± 0.8 mS/cm^2.

No difference was obtained on using the same solutions with no mannitol added, i.e., in three experiments with bilateral equilibration in Na_2SO_4 for 30 to 90 min, followed by high serosal K, both g_i and G_i increased. V_o was depolarized from −49 ± 20 to +2 ± 2 mV and, in the presence of amiloride, from −113 ± 1 to −6 ± 1 mV. Thus, both in the presence and absence of mannitol, despite the lower activity coefficient of K in SO_4 solutions, application of serosal K_2SO_4 results in abolition of V_o. It appears, however, comparing steady-state values of V_o in the presence of serosal NaCl (−75 mV; Table 1) and Na_2SO_4 (−31 mV: Table 2) that the effect of K is superimposed on a partial depolarization produced by the anion substitution.

Similar effects of high serosal K on basolateral membrane potential and conductance were obtained with NO_3-Ringer's solutions (*data not shown*, n = 4).

Transepithelial Voltage Clamping

Previous studies in K-depolarized skins involved transient voltage clamping away from the short-circuited state (e.g. Fuchs et al., 1977). It was assumed in these studies that V_t is an accurate estimate of the apical voltage V_o. We examined this point in skins depolarized by serosal K_2SO_4, clamped intermittently for periods of 20 sec at desired levels. Figure 4 shows the V_t-V_o relation in five experiments. For V_t up to 125 mV there is a linear relation between V_o and V_t (in all five experiments depicted, $r > 0.999$), with a mean slope of 0.88 ± 0.03.

Discussion

In recent years, following the influential work of Fuchs et al. (1977), many investigators of epithelial transport have used serosal solutions of high potassium concentration in an effort to minimize or largely eliminate the resistance and electrical potential gradient across the basolateral membrane. To the extent that this technique is effective, it permits assigning transcellular parameters to the apical membrane. As discussed in detail recently by Palmer (1984), attempts at K depolarization have been made for diverse purposes. Consideration of transcellular current-voltage relationships has led to the view that apical Na entry obeys the Goldman-Hodgkin-Katz "constant field" equation in the frog skin (Fuchs et al., 1977), the toad urinary bladder (Palmer et al., 1980), and the rabbit descending colon (Thompson et al., 1982), permitting the evaluation of the apical Na permeability P_{Na} and the cell Na activity. This has led to inferences concerning the apical entry process and mechanism of action of hormones (Fuchs et al., 1977; Li et al., 1982; Palmer et al., 1982; Thompson et al., 1982; Turnheim et al., 1983). The presumed minimization of apical electrical forces has facilitated fluctuation analysis of the area density and turnover rate of apical Na channels (Lindemann & Van Driessche, 1977) and the characterization of the mechanism of channel ionic fluxes (Palmer, 1982*a*,*b*). In all these applications it is unclear to what extent the values of derived pa-

rameters are affected by incomplete control of electrical potential gradients, and/or residual basolateral resistance.

Despite the widespread use of the K depolarization technique, direct evidence concerning its efficacy has been scanty. In their 1977 paper Fuchs et al. refer to "substantial, albeit indirect support," derived from the early work of Ussing and his collaborators on whole epithelia (Koefoed-Johnsen & Ussing, 1958; MacRobbie & Ussing, 1961; Ussing et al., 1965). Until recently, intracellular studies employing microelectrode techniques have been few, and the data have been conflicting. Lewis et al. (1978), working with the rabbit urinary bladder, found that in solutions with constant Cl concentration, raising the serosal K concentration above 100 mM depolarized the basolateral membrane potential by about 38 mV, as compared with a control value of 52 mV. Thompson et al. (1982) exposed the serosal surface of short-circuited rabbit descending colons to SO_4 Ringer's with 143 mM K, and found abolition of the basolateral membrane potential and marked reduction of the membrane resistance. Nagel (1977) studied skins of *Rana temporaria* and *Rana esculenta* exposed internally to isotonic K-Cl or SO_4 Ringer's solutions. Membrane potentials in short-circuited skins varied from −10 to −35 mV, irrespective of the anion, and the basolateral resistance ranged between 200 and 500 Ω cm². Tang and Helman (1983) studied 55 cells in five skins of *Rana pipiens*. Following 60-min exposure to isosmotic K_2SO_4 Ringer's solution, the membrane potential at short circuit averaged some −30 mV, and the basolateral membrane resistance had fallen to about 300 Ω cm², about one-fourth of the control value. Benos et al. (1983) utilized high serosal K concentrations in conjunction with cationic ionophores in an attempt to eliminate the basolateral membrane potential in short-circuited skins of the bullfrog, *Rana catesbeiana*. Residual potentials were −5 mV in SO_4 Ringer's, but −22 mV in Cl Ringer's. Since the latter observations conflicted with other findings, it was considered that the measurement in Cl might be artifactual. DeLong and Civan (1984) examined the effects of K_2SO_4 Ringer's solutions in split skins of *Rana pipiens pipiens,* puncturing the tissues from the serosal surface. Following 10 to 16 min of exposure to high K solutions the basolateral membrane potential in six short-circuited tissues averaged −10 mV. However, the residual potential was variable, ranging between −3 and −7 mV in three tissues, and being −18 and −19 mV in two others.

In contrast to the above-reported findings in the frog skin, we found that the basolateral membrane potential in short-circuited tissues equilibrated in high serosal K was quite regularly near-zero, irrespective of whether the predominant anion was Cl, SO_4 or NO_3, the nature of the buffer, or whether or not the solution was osmotically balanced. In 22 experiments referred to in the tables and text, the mean residual short-circuit membrane potential in high serosal K was -1 ± 1 mV, following the period of some 10 min required for achievement of a steady state. The potential remained near zero for the entire subsequent period of observation, intervals of up to about half an hour. It is not possible for us to state the basis for the discrepancy between our findings and those of others. Differences in species, hormonal influences, and other poorly defined factors could contribute. It is important to point out, however, that without continuous monitoring and care to prevent increase of R_{el} during the impalement, highly reproducible results could not be obtained. The availability of equipment which enabled us to closely follow changes in R_{el} facilitated the recognition of microelectrode artifacts which, as pointed out before, arise in almost all impalements of frog skin. Such artifacts have been recognized by other investigators, and not only in frog skin (*see* Armstrong & Garcia-Diaz, 1981), although their origin is not completely understood. They may well be the reason for the discrepancy between our findings and those previously reported.

If the basolateral membrane of frog skin exhibited almost exclusive permeability to K, increasing the serosal K activity to a value close to the cell activity should almost completely depolarize the basolateral membrane potential V_i since any contribution to V_i from pump current across this membrane would be shunted by the increased K conductance. By replacing all serosal Na by K, the serosal K activity is increased to 87 mM, in Cl (and NO_3) media. Since the cell K activity in frog skins is about 93 mM (Garcia-Diaz et al., 1985) one would expect V_i to be about 2 mV (cell negative) after this substitution, in good agreement with our results. However, when using K_2SO_4 R, the activity of K in the inner bath is only 48 mM, and, in principle one does not expect the extent of depolarization found. The reason for this discrepancy remains to be explained.

Of particular interest in this regard was the depolarization observed after anionic substitution of SO_4 (and NO_3) for Cl (compare Tables 1 and 2). DeLong and Civan (1984) also reported a depolarization in Na_2SO_4 R solutions. Possible explanations include reduction in the K conductance of the basolateral membrane (Lewis et al., 1985), reduction in the K equilibrium potential, and depression of the electrogenic Na transport mechanism. This matter is currently under investigation.

Consideration of Table 1 indicates that serosal exposure to high K in Cl Ringer's solutions reduces the cellular (sodium) current by about 55%. Since

these solutions contained nominally zero sodium, this depression of cellular current may be a consequence of uncoupling of superficial and deeper cell layers, as observed in the study of Rick et al. (1984). This possibility is supported by the insignificant depression of the short-circuit current on serosal exposure to high KCl Ringer's solutions in the toad bladder, a tissue in which there is only a single functional cell layer (Palmer et al., 1980). On the other hand, in SO_4 solutions, I_c is hardly affected by serosal substitution of K for Na (Table 2). The reason for this different response is not clear.

As has been observed by others, serosal exposure of frog skins to high K concentrations markedly increases leak conductance (Ussing et al., 1965; Fuchs et al., 1977). This has previously been attributed to stimulation of glandular activity (Share & Ussing, 1965). In addition to this possibility, we would suggest the likelihood of increased conductance of "leak" pathways such as the tight junction or increased chloride conductance in MR cells. Thus despite large increases of the basolateral conductance after raising basolateral K, values of the voltage divider ratio F_o were often well below 1, even in the presence of amiloride. Such findings have previously been found to be associated with large paracellular conductances (Nagel et al., 1983). As has been shown on theoretical grounds (Boulpaep & Sackin, 1980; Essig, 1982), increase of the junctional-to-lateral space conductance ratio leads to depression of the voltage-divider ratio. When this effect is significant, F_o becomes an unreliable index of the plasma membrane resistance ratio both in the absence and presence of amiloride (Nagel et al., 1983). This might be the result of cell swelling, with distortion of the tight junction and the contiguous extracellular pathways. It is noteworthy that only with K_2SO_4 Ringer's solutions, which Ussing et al. found not to cause cell swelling, did amiloride increase F_o to near 1 in the presence of high serosal K (Table 2).

One goal of this study was the characterization of the effect of high K solutions on the basolateral conductance. As mentioned before in Results, and discussed in detail by Nagel (1985), an unambiguous calculation of basolateral conductance is not possible at present. Although the general basis for discrepancy between g_i and G_i is unknown, several factors are pertinent under the conditions of our studies. The increase in g_p in serosal KCl R entails two possible sources of error. First there is the error in evaluating g_c as the relatively small difference in two large numbers, g_t and g_p, making the numerator in Eq. (1) imprecise. Secondly, with a highly conductive intercellular junction the voltage-divider ratio F_o will to some extent underestimate the outer membrane fractional resistance, both in the presence and absence of amiloride. When this is associated with closely similar values of F_o and F_o^A in high serosal KCl there is imprecision in the denominator of Eq. (1). For G_i on the other hand, Eq. (2) assumes linearity of the basolateral I-V relation over the full range of V_i values observed following the addition of amiloride. Although in many cases this seems to be so, often significant deviations from linearity are observed (Nagel, 1985). G_i can then only be taken as a chord conductance. Furthermore, the small change in V_i after addition of amiloride in the presence of high serosal K (some 3 to 5 mV) makes the calculation of G_i imprecise under these conditions.

Despite these uncertainties, our results show that, when calculated by either of two methods, basolateral conductance increases in the presence of high serosal [K], although the magnitude of the changes in g_i and G_i is different. It should be noted however that in the experiments with K_2SO_4-depolarized skins, using either g_i or G_i, the residual resistance of the basolateral membrane is in the range 0.5 to 1.0 KΩ cm^2. In a similar experimental situation (but with only 30 mM external Na) Fuchs et al. (1977) have calculated (Table 2, case 2) that such relatively high resistance would lead to up to 45% error in the estimate of apical membrane Na permeability P_{Na} from their fitting of I-V relations to the G-H-K equation and up to 13% error in the estimate of cell Na activity.

Whatever the absolute value of basolateral conductance, when apical conductance is characterized employing transepithelial I-V relations in depolarized skins, the pertinent consideration is that on perturbation of V_t the voltage-divider ratio should approximate unity. This was not quite the case in any of the situations examined by us. With 300 msec, 10 mV perturbations of V_t in the vicinity of short circuit, values of F_o in high K solutions ranged from 0.71 to 0.85. With intermittent 20-sec periods of voltage clamping of K_2SO_4-treated skins, with linear V_o-V_t relationships over the range 0 to 125 mV, the slope dV_o/dV_t was 0.88 ± 0.03. These findings suggest an error of about 12% in assuming $V_t = V_o$ for the K-depolarized skin under quasi-steady-state conditions. We cannot, however, predict the nature of apical I/V relationships in studies employing a rapid staircase perturbation of V_t (*see* e.g. Fuchs et al., 1977; Palmer et al., 1980; Thompson et al., 1982). Further studies will be required to clarify this issue.

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