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Saturable K^+ Pathway across the Outer Border of Frog Skin (Rana Temporaria): Kinetics and Inhibition by Cs^+ and Other Cations

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Summary. The reaction of abdominal skins of the frog species *Rana temporaria* on mucosal K^+-containing solutions was studied in an Ussing-type chamber by recording transepithelial potential difference (PD), short-circuit current (SCC) and conductance (*G*). With Na-Ringer's as serosal medium, a linear correlation between PD and the logarithm of the mucosal K^+-concentration ($[K]_o$) was obtained. The K^+-dependent SCC saturated with increasing $[K]_o$, and could quickly and reversibly be depressed by addition of Rb^+, Cs^+, and H^+. Li^+, Na^+, and NH_4^+ did not influence K^+ current. A large scatter was obtained for kinetic parameters like the slope of the PD-log $[K]_o$-line (18–36.5 mV/decade), the apparent Michaelis constant (13–200 mM), and the maximal current of the saturable SCC (6–50 $\mu A \cdot cm^{-2}$), as well as for the degree of inhibition by Cs^+ ions. This seemed to be caused by a time-dependent change during long time exposure to high $[K]_o$ (more than 30 sec), thereby inducing a selectivity loss of K^+-transporting structures, together with an increase in SCC and *G* and a decrease in PD. Short time exposure to K^+-containing solutions showed a competitive inhibition of K^+ current by Cs^+ ions, and a Michaelis constant of 6.6 mM for the inhibitory action of Cs^+. Proton titration resulted in a decrease of K^+ current at pH <3. An acidic membrane component (apparent dissociation constant 2.5×10^{-3} M) is virtually controlling K^+ transfer. Reducing the transepithelial K^+-concentration gradient by raising the serosal potassium concentration was accompanied by the disappearance of SCC and PD.

Amphibian epithelia like frog skin and toad urinary bladder absorb NaCl actively from very dilute solutions against an electrochemical gradient. Na^+ ions are assumed to cross the epithelium along a transcellular pathway [1, 3, 35, 38], whereas chloride is thought to follow mainly a paracellular path [18, 21]. Na^+-specific channels, which can also handle Li^+ ions, have been claimed to be the selective transporting structures in the apical cell membranes [11, 20]. Since transepithelial potential differences (PD) obeyed the Nernst-equation for the passive distribution of Na^+ between the mucosal solution and the cells, the

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apical membranes were referred to as "Na^+-electrodes" [35]. The saturable Na^+ uptake across these membranes can be accompanied by a rather unspecific additive cation uptake across the tight junctions. Passive movements of cations other than Na^+ and Li^+ were thought to occur through such a paracellular shunt, if a driving force was present [21, 22, 37]. The shunt pathway has the largest permeability among anions for Cl^- and among cations for K^+ [21]. Polyvalent ions like Ca^{2+}, Ba^{2+}, or La^{3+} [23, 36] and larger organic molecules or ions seem to poorly penetrate these structures unless these are made leaky, e.g., by hypertonic mucosal solutions [36].

Flux measurements [27] and permeation studies based on the observation of the transepithelial PD [15, 33] indicated an almost negligible permeability of the outer epithelial border for potassium. However, recently it has been reported that skins of the frog species *Rana temporaria* show an appreciable sensitivity for K^+ at the outer border, though this sensitivity is still small compared to that for Na^+ [14, 42].

We are presenting results here which suggest that the K^+ ions are transported through a saturable pathway in the outer border of the epithelium, which can be blocked by Cs^+, Rb^+ and protons. Saturation and selectivity of this pathway, however, seem to be time-dependent functions of the outer K^+-concentration ($[K]_o$). The localization of this K^+ pathway is discussed.

Material and Methods

The frogs (*Rana temporaria*) were kept at room temperature in tap water. The animals were double-pithed, the abdominal skins were pre-equilibrated either in Na^+-free solutions or in solutions containing Na and 50 μM amiloride to reduce active Na^+ transport (pre-equilibration time 30–45 min). Then the skins were mounted in a modified "Ussing-type" Lucite chamber. The skin surface was 3 cm^2. Voltage and current electrodes were Ag/AgCl/3-M KCl electrodes connected to the solutions by Ringer-filled agar bridges. The outer chamber compartment had a volume of only 0.85 cm^3. The mucosal solutions could be rapidly exchanged by injecting them tangentially to the skin by means of a syringe. A solution flow of 3–5 ml/sec guaranteed that the solution above the skin surface during the relatively short recording periods was well stirred. The transepithelial potential (PD) was recorded under open-circuit conditions, and the short-circuit current (SCC) was measured with a voltage-clamp device which automatically adjusted the PD to zero. Small transepithelial voltage pulses (ΔV) were imposed and simultaneous current deflections (ΔI) were measured to calculate the skin conductance (G). PD, SCC, and G were recorded with an XT-plotter (Linseis, Selb/West-Germany).

Chloride-Ringer's contained 2.5 mM $KHCO_3$, 1 mM $CaCl_2$ and 115 mM of the chloride of the main cation, e.g., choline, Na, K or Cs (Chol-R, NaCl-R, KCl-R, CsCl-R). The pH was adjusted with HCl to 7.4. pH-titration was done with solutions which were

acidified with HCl. No special buffers for acid pH were used since during the very short experiments (contact time with acid solutions below 10 sec) the solution flow was constantly held at 5 ml/sec and therefore guaranteed a stable pH value of the mucosal medium. For some experiments gluconate-Ringer's was used containing 1 mM Ca-gluconate, 110 mM Na^+-gluconate (NaG-R) or K^+-gluconate (KG-R) and 5 mM Tris as buffer (pH 7.4). The serosal solution was mostly air-bubbled NaCl-Ringer's (with chloride solutions, pH 8.4), and sometimes Na^+-gluconate-Ringer's. Short-time experiments for K^+-Cs^+-competition studies and pH titration were done in such a way that skin exposure to K^+ was never longer than 30 sec. In these experiments, K^+-free solutions contained choline or Na^+ (with 50 μM amiloride) instead of K^+. In all solutions the concentration sum of the monovalent cations was held constant (e.g., $[Chol]_o + [K]_o = 117.5$ mM).

Results

1. Kinetics of the Transepithelial K^+ Movement and Time-Dependent Selectivity Change

With Na^+-Ringer's as serosal medium, a sudden change from mucosal choline Ringer's (containing 2.5 mM K^+) to potassium Ringer's (117.5 mM K^+) resulted in an instantaneous rise of the transepithelial open-circuit potential (PD) from values of practically zero mV usually up to 30–40 mV, serosal side positive. Figure 1 shows that in the investigated range of mucosal K^+ concentrations ($[K]_o$) the relationship between the logarithm of $[K]_o$ and the PD for a representative skin is

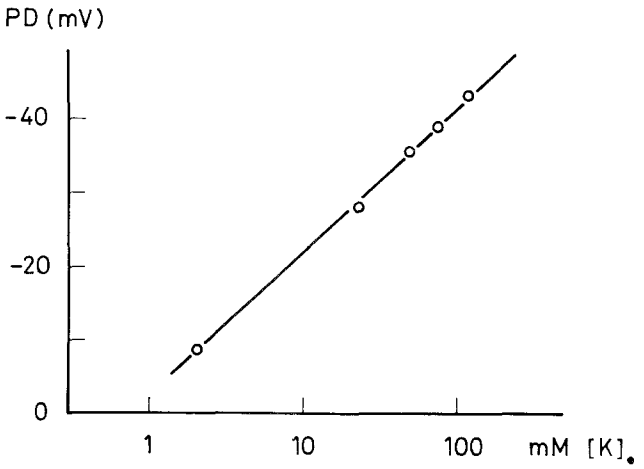


Fig. 1. Relationship between the transepithelial potential difference (PD) and the logarithm of the mucosal K^+-concentration $[K]_o$. PD is negative on the mucosal side of the skin. Solutions: serosal – NaCl-R; mucosal – chloride-Ringer's with sum of K- and choline concentration always being 117.5 mM

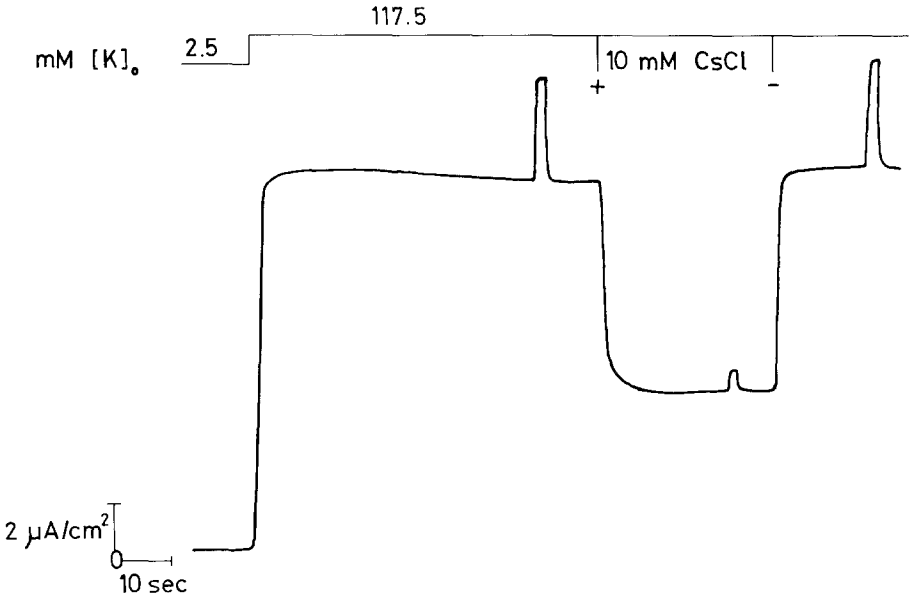


Fig. 2. Time course of short-circuit current (SCC) after a substitution of mucosal choline chloride Ringer's by KCl-R and inhibitory effect of 10 mM CsCl in the outer solution. The vertical bars are current responses when the voltage was clamped for some seconds from zero to 10 mV, outside positive. Serosal solution: NaCl-R

linear. However, the maximal PD obtained with $[K]_o = 117.5$ mM (PD_{max}), and also the slope of the line in the semilogarithmic plot showed in different skins a large scatter, the former ranging from 15.5 to 36 mV, the latter from 18.2 to 36.5 mV/decade. A slope of 58 mV per 10-fold $[K]_o$ change, as would be expected for an electrode-like behavior of the outer skin border, was never observed. Addition of amiloride or the use of an impermeant anion (gluconate) practically did not change the PD-$[K]_o$-relationship.

With mucosal KCl-Ringer's, the clamping of the PD to zero mV resulted in a positive inward directed short-circuit current (SCC). A sudden change from mucosal choline to potassium Ringer's resulted in a very rapid rise of the SCC. A typical skin response is seen in Fig. 2. The slightly positive SCC with choline (0.4 μA/cm²) reached a plateau value of 13.1 μA/cm² 5 sec after the solution change. At the same time the transepithelial conductance rose from 0.17 to 0.38 mmho/cm². A similar change of the SCC was never observed after substitution of choline by K^+-free solutions containing only CsCl or NaCl with 50 μM amiloride. The addition of 10 mM CsCl to the solution resulted in a sudden decrease of the SCC to about 50% of the original value, and a sharp conductance

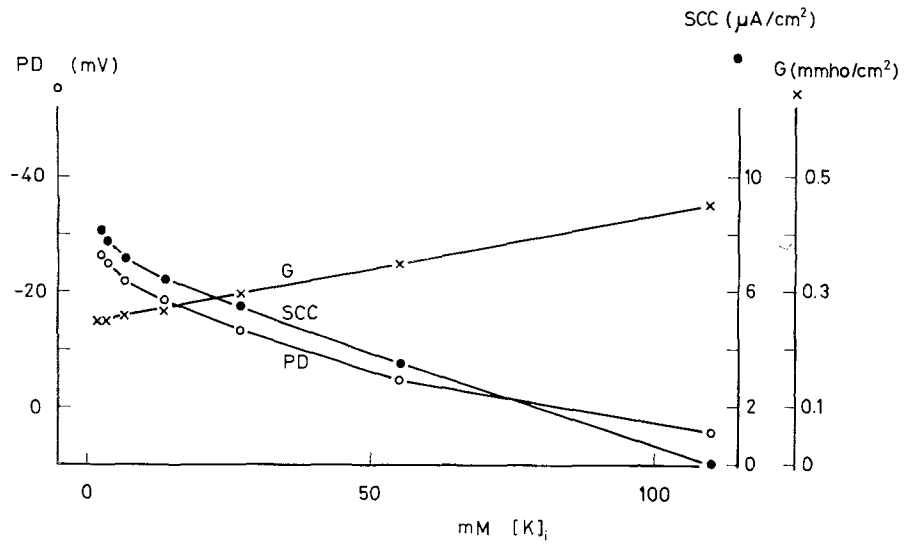


Fig. 3. Change of transepithelial potential difference (PD) (○), short-circuit current (SCC) (●), and conductance (G) (×) as a function of the serosal K^+ concentration $[K]_i$. Solutions: mucosal – KG-Ringer's with 50 μM amiloride; serosal – gluconate Ringer's, the sum of K^+ and Na^+ always being 110 mM

drop from 0.38 to 0.08 mmho/cm^2 (conductance proportional to the length of the vertical bars being current responses at a short voltage pulse of +10 mV, outside positive). The fast SCC rise, the surprisingly rapid action of Cs^+, and the quick reversibility suggest that the K^+-specific pathway and the site of the inhibitory Cs^+ effect must be located next to the outer skin border.

The recorded SCC does not seem to be depressed by Na^+ backflux from the serosa because a negative current could not be observed when the mucosal solution was Na^+ free and contained either choline- or Cs^+-Ringer's. Figure 3 shows an experiment where Na^+-gluconate was gradually replaced by K^+-gluconate in the serosal solution. This substitution resulted in a decrease of the K^+-dependent PD and SCC until they disappeared when the transepithelial chemical K^+-gradient vanished. From this experiment it may be concluded that the SCC is probably passive. A large increase in conductance was observed.

In most skins the initially reached SCC began to increase again after 20–30 sec. This late current phase leads to a second plateau which can be more than 30% higher than the initial one, and is reached in 3–5 min. Figure 4 shows how PD, SCC and G change after a substitution of mucosal potassium for choline Ringer's. A concomitant PD fall can be seen together with an increase in SCC and conductance. This very typical

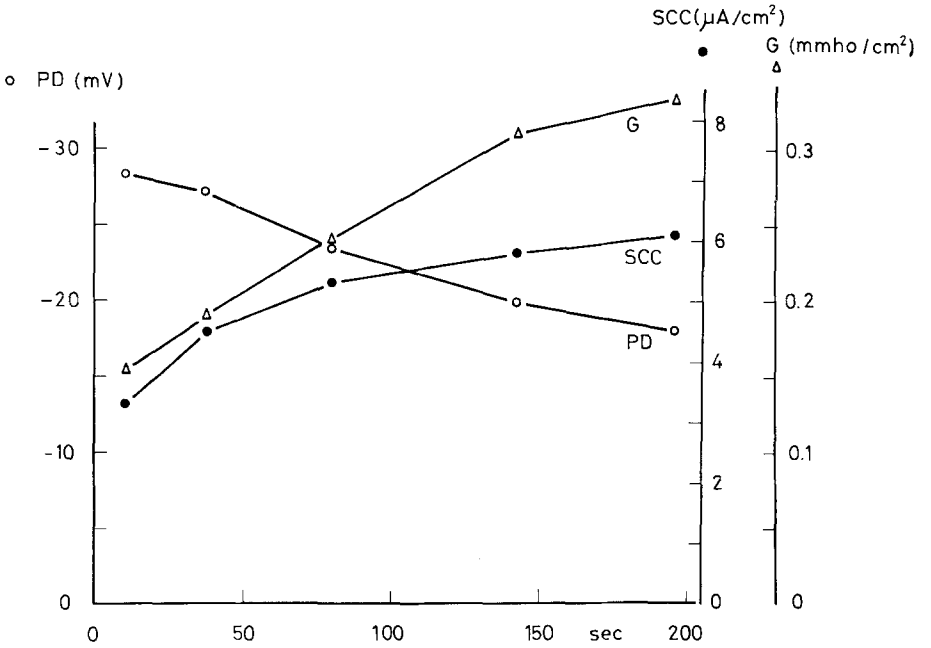


Fig. 4. Time-dependent change of potential difference (PD), short-circuit current (SCC), and conductance (G) after a mucosal choline-potassium substitution. The abscissa indicates the time after the solution change when the data were read off. Solutions: serosal – NaCl-R; mucosal – KCl-R

behavior after contact with mucosal K^+ may have its origin either in a delayed "opening" of a second K^+ pathway or in a change of the K^+-selective structure from an "early" to "late state" with higher conductance. It will be shown below that the latter probably is responsible for the rise of the "late phase".

The SCC shows saturation with increasing $[K]_o$ (Fig. 5*a*). However, comparing the maximal currents (SCC_{max}) and K_m for different skins, a similar scatter of these parameters is seen as for the maximal PD and the slope of the PD-log $[K]_o$-relation. The maximal SCC (extrapolated for $1/[K]_o \to 0$ in double reciprocal plots of SCC *vs.* $[K]_o$, like Fig. 5*b*) ranges from 6.3 to 50.1 μA/cm², whereas the K_m values vary between 13.5 and 200 mM. This scatter can be expected if the SCC values are recorded at different times after bringing the mucosal border into contact with K^+. Indeed, the saturation behavior changes when the SCC data are read off either in the early or late phase of contact time with K^+. Figure 5*a* shows that data recorded in the early phase (5 sec after contact with K^+) yield smaller maximal currents and K_m's than data from the late phase (after 3 min contact with K^+). The corresponding double recipro-

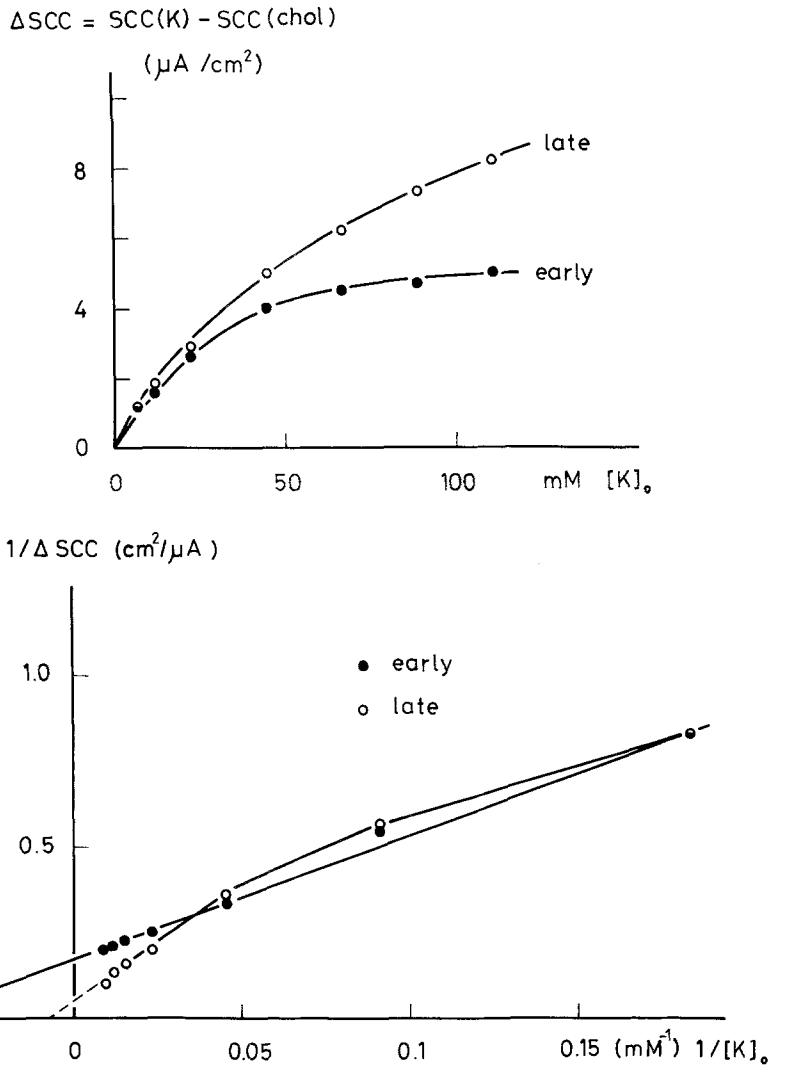


Fig. 5. (*a*): Increase of the short-circuit current (ΔSCC) after a mucosal choline-potassium substitution as function of the mucosal K^+ concentration $[K]_o$. The curve labeled "early" shows data recorded only 5 sec after the solution change. The curve labeled "late" is obtained 3 min after the substitution in the same skin preparation. (*b*): Double reciprocal plot of the data of *a*. Solutions: serosal – NaG-R; mucosal – gluconate Ringer's with 50 μM amiloride, the sum of K^+ and Na^+ always being 110 mM

cal plot (Fig. 5*b*) reveals an almost perfect linear relationship for the early phase, whereas for the late phase a curve is obtained. Such a shift easily explains the large variation of SCC_{max}, K_m, PD_{max} and the slope of the PD-log $[K]_o$-line. For the examination of kinetics and effects of drugs and ions, it is therefore important to study the skin response in

both phases of K^+ conductivity. This will be done in detail for the inhibitory action of Cs^+ on the K^+ current.

2. *Inhibition of K^+ Current*

In this chapter the influence of monovalent cations (Li^+, Na^+, Rb^+, Cs^+, NH_4^+ and protons) on the K^+ current will be studied. The chlorides of these cations were added to KCl-Ringer's on the mucosal side of the epithelium in a concentration of 10 mM. In Li^+- and Na^+-containing solutions 50 μM amiloride were present, which is known to block Li^+- and Na^+-diffusion through the Na^+ channels in the mucosal membrane [8, 26]. Of the tested cations, only Rb^+, Cs^+ and H^+ inhibited K^+ currents. Figure 6 shows current-voltage relationships with NaCl-Ringer's as serosal and different mucosal media. At short-circuit conditions the K^+ current turned out to be always less than 50% of the steady-state Na^+ current. In presence of 10 mM LiCl and NaCl in 50 μM amiloride containing KCl-Ringer's, the K^+-dependent $I-V$ curve was not significantly changed. However, by RbCl and even more by CsCl, the

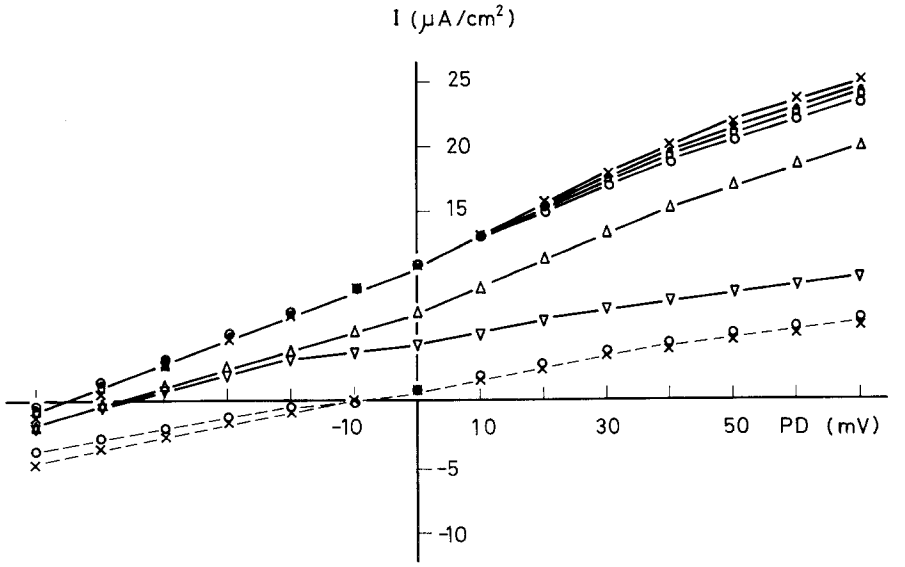


Fig. 6. Current-voltage relationship of a skin. Positive inward currents increase with increasing serosal negativity (PD positive). Mucosal solutions: KCl-R (——×——); KCl-R with 10 mM LiCl and 50 μM amiloride (——○——); KCl-R with 10 mM NaCl and 50 μM amiloride (——●——); KCl-R with 10 mM NH_4Cl (——□——); KCl-R with 10 mM RbCl (——△——); KCl-R with 10 mM CsCl (——▽——); choline chloride Ringer's (··· × ···); CsCl-Ringer's (··· ○ ···). Serosal solution: NaCl-R

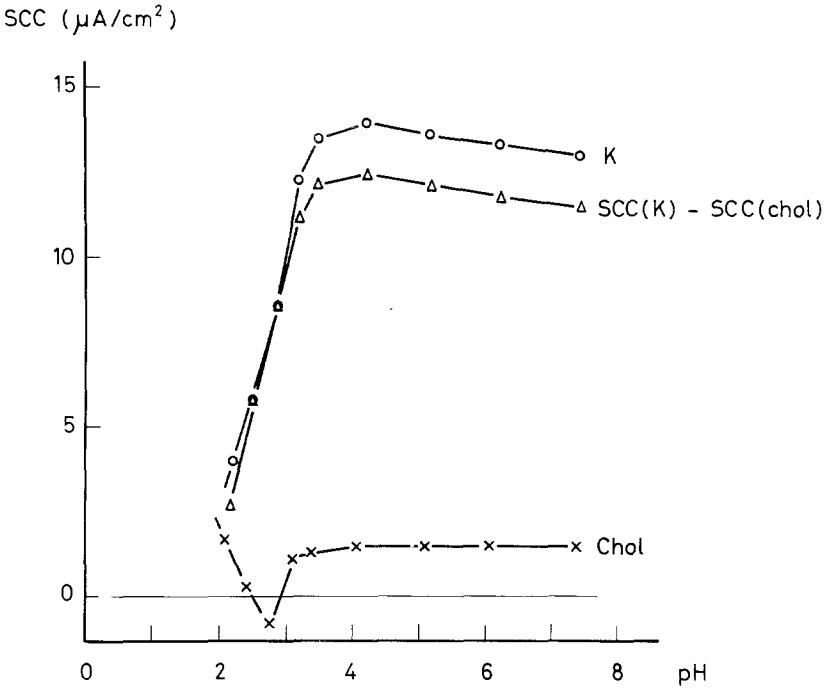


Fig. 7. Short-circuit current as a function of the mucosal pH value with choline Ringer's (×) or KCl-R (○) as mucosal solution. Subtraction of the data of the lower curve from the current values of the upper one yields the middle trace (△). The pH was adjusted with HCl. Serosal solution: NaCl-R, pH 8.4

current was appreciably reduced and the conductance likewise decreased. Only small currents and conductances were obtained with choline chloride- and CsCl-Ringer's as mucosal solutions. Figure 6 shows that of the alkali cations only Rb^+ and especially Cs^+ can inhibit K^+ current.

A similar reduction of the K^+ current can be obtained when the mucosal solution is acidified (Fig. 7). "Titrating" the outer skin border with HCl resulted in a slight increase of the K^+-dependent SCC, until at pH < 3.5, the SCC dropped. The experiments were done with short-time exposure to K^+-containing solutions at different pH, thus avoiding the rise of the "late state" of K^+ pathway. The lower curve was recorded with choline on the mucosal side. Here, the initial slightly positive SCC decreases below pH 3, becomes somewhat negative at pH 2.7, and rises steeply at pH <2.5. This phenomenon has already been observed in Na^+-containing solutions (with and without amiloride) and was interpreted as a sign of H^+ influx along the transepithelial gradient [40, 41]. The middle curve is obtained after subtraction of the unspecific titration curve (choline) from the upper one and represents the true

titration curve of the K^+-selective pathway in the outer border of the skin. In a number of experiments with K^+ on the mucosal side, the decrease of the SCC below pH 3 was masked by an increase of the unspecific current already at pH 3. Thus the recorded SCC showed a minimum between pH 2–3. After subtraction of the unspecific current, a curve similar to the middle curve in Fig. 7 was obtained. All pH effects were reversible, though the recovery needed some minutes in the case of very high acidity. The half maximal SCC was reached at pH 2.6, which indicates an apparent H^+-dissociation constant of the titrated site of 2.5×10^{-3} M.

In summary, it must be stated that the outer border of the skin of *Rana temporaria* is able to take up K^+ selectively through pathways which cannot be blocked by Li^+-, Na^+- and NH_4^+-ions, but by Rb^+ and Cs^+ as well as by protons. A negatively charged group with acid character seems to control the uptake of K^+ into the epithelium. In the following section, the effect of Cs^+ as the most potent blocker at physiological pH will be studied in detail.

3. The Nature of the Inhibition of K^+ Current by Cs^+ Ions

Figure 2 showed the fast and reversible inhibitory influence of Cs^+ on the K^+-dependent SCC. A dose-response curve of the blocking action of Cs^+ is shown in Fig. 8. Adding increasing amounts of CsCl to the mucosal K^+ solution yields an *S*-shaped current decrease in a semi-logarithmic representation. The upper curve represents the SCC when Cs^+ was added during the late phase (5 min contact with K). The lower curve was obtained after choline-K^+ substitution during the early phase (15 sec contact with $K^+ \pm Cs^+$). Not only were the SCC values higher in the late phase, as expected, but also the half-maximal inhibition (i.e., the apparent Michaelis constant of Cs^+, (K_{Cs})) increased from 6.6 to 8.5 mM. This result indicates that the degree of inhibition by Cs^+ is different in the "early" and "late state" of the K^+ pathway.

We observed in many skins that the percentage current depression by 10 mM Cs^+ was smaller with increasing contact time of the skin to the mucosal K^+ solution. An extreme example is shown in Fig. 9. After about 1 hr, the SCC was no longer influenced by Cs^+. Possible mechanisms, e.g., the selectivity loss of the K^+-selective structures or the opening of an unspecific parallel pathway, underlying the transition of the skin from early to the late state will be discussed in the discussion section of this paper.

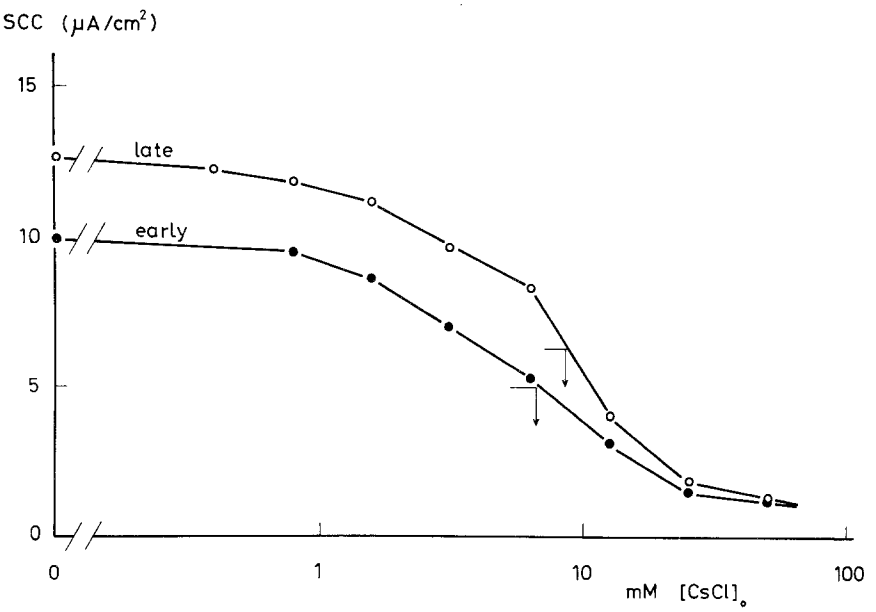


Fig. 8. Short-circuit current (SCC) as a function of the mucosal CsCl concentration. The curves were obtained after not more than 15 sec (early) and 5 min (late) contact time with mucosal K^+ and Cs^+. Arrows indicate the half-maximal values. Solutions: serosal – NaCl-R; mucosal – KCl-R with CsCl

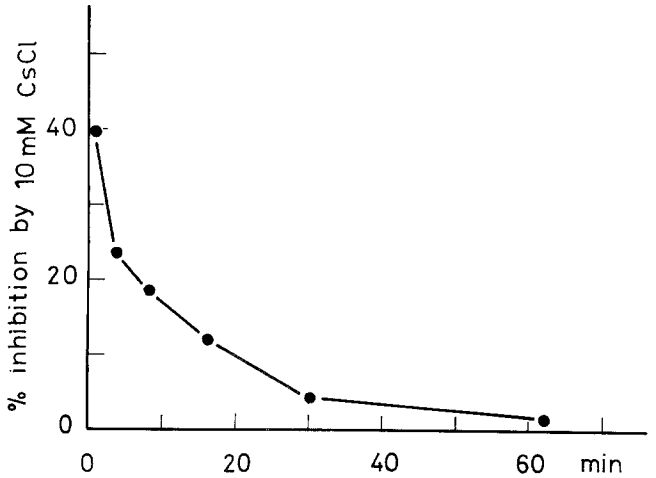
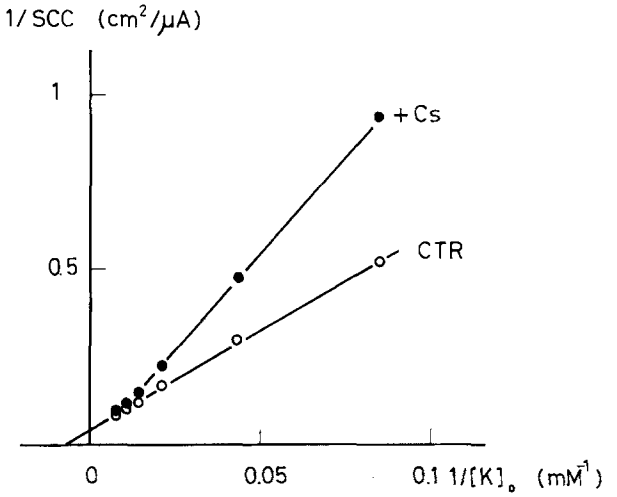


Fig. 9. Percentage depression of the short-circuit current (SCC) after addition of 10 mM CsCl as a function of skin contact time to potassium. Solutions: serosal – NaCl-R; mucosal – KCl-R

To investigate the nature of the inhibition of the K^+ current by Cs^+, dose-response curves were recorded with and without 10 mM CsCl in the K^+-containing solutions some minutes after choline-K^+ substitution, i.e., in the late phase. In Fig. 10*a*, the control (CTR) in a double reciprocal


1/SCC (cm²/μA)
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0.05
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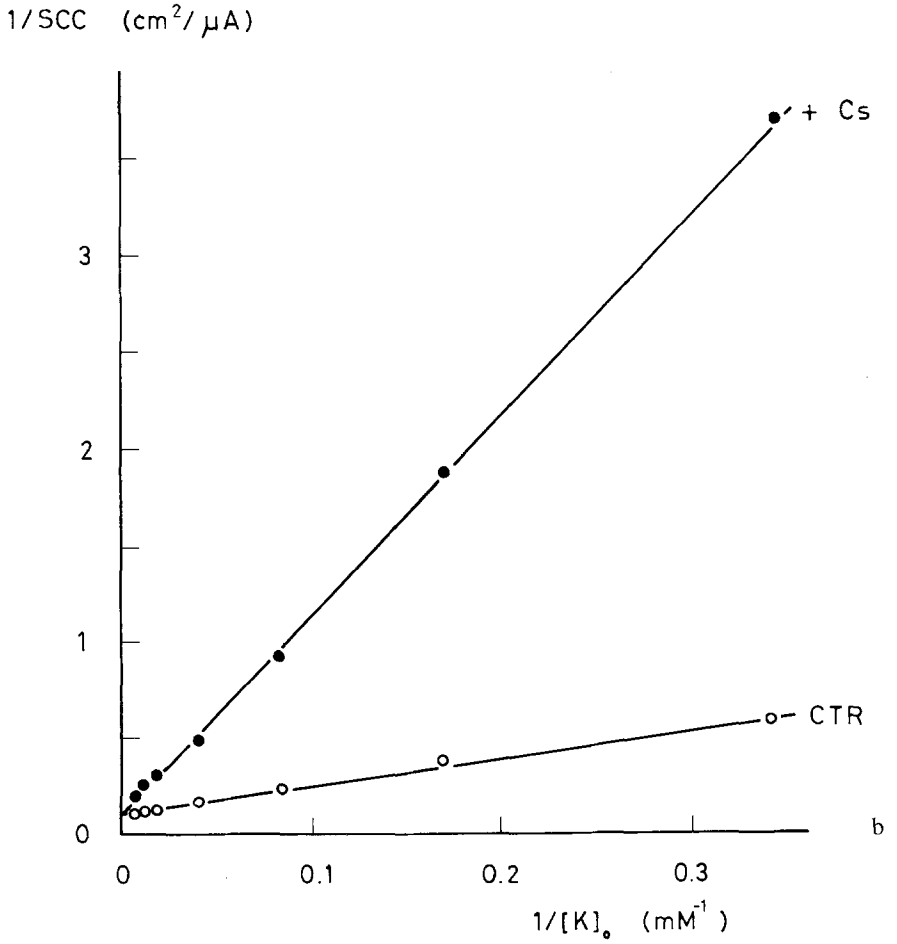
1/SCC (cm²/μA)
3
2
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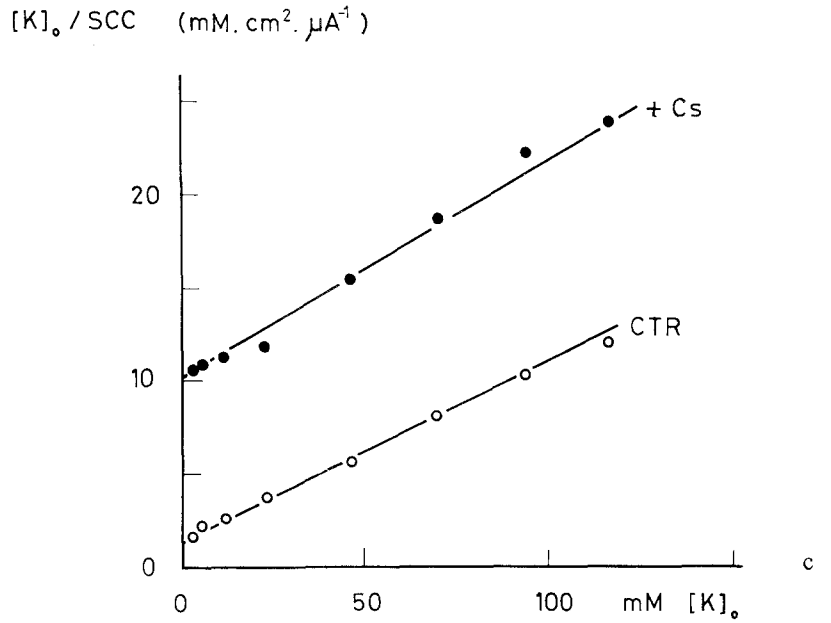

Fig. 10. (*a*): Double reciprocal plot of the K^+-dependent short-circuit current (SCC) as a function of the mucosal K^+ concentration $[K]_o$ without (CTR) and with 10 mM CsCl in the outer solution (+Cs). The SCC values were obtained in the late current phase after more than 5 min contact time with mucosal potassium. Solutions as in Fig. 1. (*b*): "Lineweaver-Burk" diagram as in *a*. The SCC values were, however, obtained in the early current phase with less than 30 sec contact time to mucosal potassium. Solutions: serosal – NaG-R; mucosal – gluconate Ringer's with 50 μM amiloride, the sum of K^+- and Na^+-concentration being always 110 mM. (*c*): Data of *b* plotted in a different way (*see* text)

plot of a dose-response curve is a straight line. If Cs^+ were a competitive inhibitor of K^+ current, the CTR-line should have been rotated counterclockwise around the ordinate intercept in the presence of Cs^+. The addition of Cs^+ did result in a counterclockwise rotation; however, at higher $[K]_o$, the two curves seemed to melt into one another. This can be understood because the degree of inhibition decreases after long time exposure to high $[K]_o$. On the other hand, Fig. 10 shows that, in addition to the length of exposure time to high $[K]_o$ solutions (Fig. 9), the amount of K^+ in the solution also contributes to the transition from the "early" to the "late state" of the K^+ pathways.

To study the true nature (Fig. 10) of the Cs^+ inhibition, experiments were carried out with short time exposure to K^+-containing solutions. A Lineweaver-Burk diagram of data recorded in that way is shown in Fig. 10*b*. The short time experiments show that Cs^+ ions are competitive inhibitors of the K^+ current in the "early state" of the K^+ pathway: the

straight control line (CRT) is rotated around the ordinate intercept counterclockwise; the maximal current is unchanged, but the apparent Michaelis constant increases from $K_m = 14$ mM to $K_m^* = 106$ mM in the presence of 10 mM CsCl. Although the double reciprocal plot already gives the desired information, another graphical analysis of the data was carried out. Rearranging the equation for a saturable K^+ current.

$$SCC = SCC_{max} \cdot \frac{[K]_o}{[K]_o + K_m} \tag{1}$$

(with SCC_{max} as maximal current and K_m as the apparent Michaelis constant) gives another linear relationship:

$$\frac{[K]_o}{SCC} = \frac{K_m}{SCC_{max}} + \frac{1}{SCC_{max}} \cdot [K]_o. \tag{2}$$

Plotting $[K]_o/SCC$ against $[K]_o$ (Fig. 10*c*) results in a straight line, but the experimental data do not accumulate near the origin as in a double reciprocal diagram. The slopes of the two lines are identical and equal to the inverse maximal current, which is not changed in the presence of Cs^+. The upwards shift of the ordinate intercept K_m/SCC_{max} with Cs^+ means an increase of K_m to a higher value K_m^* as expected from the action of Cs^+ as a competitive inhibitor in the "early state" of the K^+ pathway.

Discussion

It has been shown that the abdominal skin of *Rana temporaria* is able to take up potassium ions from the mucosal solution. A transepithelial PD (mucosal side negative towards serosa) and a saturating (inward directed) SCC, both dependent on the presence of K^+ in the mucosal medium, are the sign of a specific K^+ transfer across the mucosal border. The very rapid and fully reversible inhibition by mucosal Cs^+ ions indicates that the K^+ selective structure is located near the apical border of the epithelium. Long time exposure to high $[K]_o$ increased K^+ current, but considerably decreased the originally competitive inhibitory effect of Cs^+. Of the other alkali ions, only Rb^+ inhibited K^+ current whereas Li^+, Na^+ and the pseudo-alkali NH_4^+ did not. Protons at concentrations of more than 1 mM where also found to block K^+ current. Reducing the transepithelial K^+-concentration gradient was likewise decreasing K^+ current. Amiloride did not influence all observed K^+-dependent data.

There is no agreement in the literature about K^+ movement across the outer border of amphibian epithelia. Some authors found K^+-dependent diffusion potentials and short-circuit currents in normal epithelia [28, 33], only with mucosal hypertonicity [7] or after inhibition of the active Na^+ transport by ouabain [21]. Conductance measurements revealed an increase of conductance with increasing $[K]_o$ [12, 19], and a saturating K^+ conductance was even observed for the skin of *Rana esculenta* [19]. Rather low K^+ permeabilities (P_K) were found which were usually 5–10% of the mucosal Na^+ permeability [15, 21, 33, 37]. A negligible mucosal P_K has even been reported [9, 27, 35].

For instance, in the frog species *Lepodactylus ocellatus* a change from low Na^+-containing to high mucosal K^+ solutions showed practically no K^+-dependent SCC [5]. A K^+ efflux to the mucosal side, proportional to the Na^+ uptake has been described [29]. Inhibitory effects of mucosal K^+ on active Na^+ transport have also been described [12, 22]. Such a clear saturating transepithelial K^+ movement which we have found only in the skin of *R. temporaria*, but not with *R. esculenta* and the toad urinary bladder (W. Zeiske and W. Van Driessche, *unpublished*), had never before been observed.

1. Kinetics of the Conductive K^+ Pathway in the Outer Border

K^+movements across the outer skin border were thought to occur mainly along a paracellular pathway, i.e., via the zonulae occludentes and the tight junctions [21, 37]. However, in a few studies, the existence of K^+-selective structures in the apical cell membranes has been reported. Microelectrode investigation of the frog skin excitability (*R. esculenta*) revealed K^+ channels which were equally permeable for Na^+ [19]. Another group recently found Ba^{2+}-blockable, K^+-selective structures in the apical cell membranes (*R. temporaria*) [14]. No dependence of K^+ selectivity on the moulting cycle of the skin was found. However, a transient (lasting only some hours) K^+ sensitivity of the mucosal skin border has been observed after the natural moult process [17], and also after a shedding induced with aldosterone [9].

The K^+-selective structures could be blocked with Ba^{2+} [9, 14], and even a short-lasting sensitivity towards amiloride was found, suggesting a transition of the former K^+ channels in the serosal membranes into Na^+-specific channels of the new outermost living cell layer after the moult [17]. In our experiments, amiloride did not have any influence on

K^+-dependent parameters. We also detected an inhibitory Ba^{2+} effect on K^+ current (*unpublished*), but preferred to study intensively the inhibitory effect of Cs^+ ions. Both ions are claimed to block "K^+ channels" in other tissues [16, 34]. We showed that in the "early state" of the K^+-selective structure, the inhibitory Cs^+ ions were competitive with K^+ ions. The change from the "early" to the "late state" of the K^+ pathway was accompanied by a decrease of the Cs^+-blocking effect. Protons are known to inhibit apical Na^+ influx in frog skin [40, 41]. Proton titration of the skin led to the conclusion that the negative group which seems to control the K^+ movement must have an apparent proton dissociation constant of 2.5×10^{-3} M. This value suggests the involvement of a carboxylic or phosphate group which is also thought to control Na^+ uptake in frog skin [41].

At least in the early state a very specific apical K^+ pathway can be characterized by typical $[K]_o$-dependent changes of transepithelial PD, short-circuit current, and conductance. Though the PD-log-$[K]_o$ relation is linear with a slope of much less than 58 mV per 10-fold change of $[K]_o$, this must not be taken as an indication of a – possibly shunted – K^+ diffusion potential across the outer skin border. Though the outer border was claimed to be a perfect "Na^+-electrode" [35], this view has been challenged during the last years [10, 25, 30]: Linear PD-log $[Na]_o$ plots showed about the same slope as was found in the present experiments with mucosal K^+. To explain the observations, electrical interdependences of the two cellular membranes were presumed [10, 30]. An equivalent circuit analysis [32] revealed the importance of the ratio of trans- and paracellular resistances for sign and behavior of the transepithelial PD. Since possibly the same complicating factors are also true for K^+, the PD cannot be seen as a reliable indicator of a diffusion potential [25]. It may only be stated at the present time that the apical skin sensitivity for K^+ results in a concentration-dependent PD. At the same time the K^+-dependent increase in epithelial conductance and the inward directed SCC point towards a rather high K^+ permeability of the apical border.

2. *Do K^+ Ions Use Trans- or Paracellular Paths?*

The K^+-selective structures might be membrane-bound water filled pores, as suggested for the passage of Na^+ ions [11, 20], or the zonulae occludentes in the paracellular pathway [6, 21]. In any case they must

allow a certain degree of selectivity [21] and the existence of a saturable SCC as well as the inhibition by Cs^+, Rb^+ and H^+. Especially the change from an "early" to a less specific "late state" must be conceivable with the properties of the K^+ uptake.

Our experiments showed that a decrease of the transepithelial K^+-concentration gradient by augmenting serosal potassium abolished the SCC. This result is easily understood if the K^+ current was passive and paracellular. In the short-circuited state, also the transcellular K^+ movement would be energetically downhill since at even low $[K]_o$ the apical electrical gradient (cell negative) favors K^+ diffusion into the cells against the chemical gradient [31], whereas the basolateral chemical K^+ gradient favors K^+ diffusion from cells into the interspaces against the electrical basolateral gradient (the negative transmembrane potential step at short-circuit conditions [4, 24] is symmetrical but opposite at the two membranes). Under short-circuit conditions, a net transcellular K^+ movement would therefore not need any active driving forces.

If membrane-bound apical K^+ pathways existed, the "long-time effect" of high $[K]_o$ could be due to:

1) an increase of the number of K^+-selective structures,

2) the opening of an unspecific paracellular pathway, or

3) an increase of the permeability of the existing K^+ pathways caused by decrease of the selectivity.

Equivalent circuit analysis would offer an explanation if the K^+-dependent PD were assumed to be a "shunted K^+-diffusion potential". The first mechanism would increase SCC and *G* but leave the PD and the inhibitory Cs^+ effect unchanged, which is not consistent with our observations. The second possibility would explain the observed PD drop and conductance increase. However, equivalent circuit analysis shows that the SCC must then remain unchanged, which was not observed experimentally. The third hypothesis could easily explain the alterations of PD, SCC and *G*. The selectivity decrease during the long time exposure to mucosal potassium would result in the observed decreased inhibitory Cs^+ effect (Fig. 8 and 9). Whatever the exact nature of a "widening" of the K^+-selective structures would be, the change of specific to less specific structures would create an additional ion diffusion pathway, shunting a K^+-diffusion potential.

The extracellular shunts prefer K^+ over other monovalent cations [21], but seem to exclude polyvalent ions [23] and larger molecules. The long time effect evoked by high $[K]_o$ led to a PD fall and an increase of SCC and *G*. At the same time the inhibition by Cs^+ was decreased. In

case of a paracellular K^+ diffusion through selective zonulae occludentes, a local osmotic mechanism as origin of the transition from the "early" to "late state" is imaginable. Hypertonic mucosal KCl is able to induce the formation of blisters in the tight junctions [2], a result of water accumulation after an increased salt transfer from the mucosal solution into the junction.

Fluctuation analysis of the K^+-dependent SCC suggested the presence of K^+-specific structures at the apical skin border of *R. temporaria*, but led first to the hypothesis of a transcellular [39], later of a paracellular [43] K^+ movement. While the latter suggestion was in conformity with a proposed selective, fluctuating tight junction [6], recently reported microelectrode investigations make it likely that the K^+-selective structures are rather localized in the apical cell membranes [14]. These specific pathways could be remaining "K^+-channels" of the former basolateral membranes which are transformed into more Na^+-specific membranes during the moult (*see also* 9, 17). In contrast to findings in other frog species, the density of apical K^+ pathways would then be very high.

The negative potential step across the apical membrane is even larger with impermeant cations at the mucosal side and could actually be responsible for a rectifying force even in the open circuited skin [13, 24]. The very low ion concentrations of the natural environment of the frogs should lead to an even more negative potential well which could easily prevent a K^+ loss through the apical membranes. A geometrical rectification of membrane-bound K^+ pathways is also imaginable.

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