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The Interaction of "K^+-like" Cations with the Apical K^+ Channel in Frog Skin

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Summary. The apparent permeability of the apical K^+ channel in the abdominal skin of the frog (*Rana temporaria*) for different monovalent cations was tested by comparing the short-circuit current (SCC) obtained after imposition of serosally directed ionic concentration gradients. Furthermore, the SCC was subjected to noise analysis. Of various cations tested, only the "K^+-like" ions NH_4^+, Rb^+ and Tl^+, besides K^+, were found to permeate the apical K^+ channel, as reflected by SCC- and fluctuation analysis: (*i*) The SCC could be depressed by addition of the K^+-channel blocker Ba^{2+} to the mucosal solution. (*ii*) With the K^+-like ions (Ringer's concentration), a spontaneous Lorentzian noise was observed. Plateau values were similar for K^+ and Tl^+, and smaller for NH_4^+ and Rb^+. The corner frequencies clearly increased in the order $K^+ < NH_4^+ < Tl^+ \ll Rb^+$. The SCC dose-response relationships revealed a Michaelis-Menten-type current saturation only for pure K^+- or Tl^+-Ringer's solutions as mucosal medium, whereas a more complicated SCC behavior was seen with Rb^+ and especially, NH_4^+. For K^+-Tl^+ mixtures an anomalous mole-fraction relationship was observed: At low $[Tl^+]/[K^+]$ ratios, Tl^+ ions appeared to inhibit competitively the K^+ current while, at high $[Tl^+]/[K^+]$ ratios, Tl^+ seemed to be a permeant cation. This feature was also detected in the noise analysis of K^+-Tl^+ mixtures. Long-term exposure to mucosal Tl^+ resulted in an irreversible deterioration of the tissue. The SCC depression by Ba^{2+} was of a simple saturation-type characteristic with, however, different half-maximal doses ($NH_4^+ < K^+ < Rb^+$). Ba^{2+} induced a "blocker noise" in presence of all permeant cations with corner frequencies that depended on the Ba^{2+} concentration. A linear increase of the corner frequencies of the Ba^{2+}-induced noise with increasing Ba^{2+} concentration was seen for NH_4^+, Rb^+ and K^+. With the assumption of a pseudo two-state model for the Ba^{2+} blockade the on- and off-rate constants for the Ba^{2+} interaction with the $NH_4^+/Rb^+/K^+$ channel were calculated and showed marked differences, dependent on the nature of the permeant ion. The specific problems with Tl^+ prevented such an analysis but SCC- and noise data indicated a comparably poor efficiency of Ba^{2+} as Tl^+-current inhibitor. We attempted a qualitative analysis of our results in terms of a "two-sites, three-barriers" model of the apical K^+ channel in frog skin.

Key Words frog skin · K^+ channel · selectivity · gating · blockage · single filing

Introduction

For many years frog skin has been an extremely valuable model to study ionic movements in epithelia, especially transcellular Na^+ transport (e.g. Ussing & Windhager, 1964; Lindemann & Voûte, 1976; Ussing, 1978; Zeiske, 1978). Since the past four years frog species *Rana temporaria* was recognized to have K^+-specific channels in the apical skin membrane (Zeiske & Van Driessche, 1979; Nagel & Hirschmann, 1980) in parallel to the well-described Na^+ channels. This finding again opened a pathway for studies of transcellular K^+ movements in this easy to handle epithelium. Frog skin might be, in this respect, a suitable model for K^+-transport systems in other, less easily approachable tissues as e.g. the kidney tubule (Giebisch, 1981).

Noise analysis of ionic currents turned out to be of great help in characterizing apical K^+ pathways in gallbladder (Van Driessche & Gögelein, 1978), amphibian stomach (Zeiske, Van Driessche & Machen, 1980), mammalian colon (Wills, Zeiske & Van Driessche, 1982) as well as in larval (Zeiske, Hillyard & Van Driessche, 1982) and adult frog skin (Van Driessche & Zeiske, 1980*a*, *b*). Noise analysis was also appropriate to study K^+ channels in basolateral membranes of epithelia (Van Driessche et al., 1981). Apart from functional aspects, the microscopic properties of epithelial K^+ channels are of primary interest, not only for comparative reasons in the field of epithelial physiology but also for comparison with properties of the well-studied K^+ channels in excitable tissues. Here we describe investigations concerning the ionic selectivity of the apical K^+ channel in frog skin (*Rana temporaria*) as suggested by (*i*) steady-state analysis of the macroscopic short-circuit current, and (*ii*) the current-noise analysis. Our results indicate some striking similarities of the apical K^+ channel in frog skin when compared to K^+ channels in excitable membranes. Especially, a dependence on the nature and concentration of the permeant ions (Tl^+, K^+, Rb^+, NH_4^+) seems to exist for channel gating, permeability, and blockage of the channel by Ba^{2+} ions.

Materials and Methods

Rapid Flow Experiments

Abdominal skin of the frog species *Rana temporaria* was mounted in an Ussing-type chamber (3 cm^2) made from Lucite®, and designed to allow rapid solution changes at the mucosal side of the epithelium. (For details *see* Zeiske, 1978; Zeiske & Van Driessche, 1979.) This method is certainly slower than the fast-flow equipment developed by Lindemann, Gebhardt and Fuchs (1972) which allowed a 95 percent exchange within 25 msec. Nevertheless we are able to observe current responses faster than 1 sec, elicited by changes in mucosal solution composition (*cf.* Fig. 1 this paper; Fig. 1 in: Van Driessche and Zeiske, 1980*b*; Fig. 4 in: Zeiske, 1978). The skin was short-circuited, and the short-circuit current (SCC) was recorded for various mucosal conditions. During the rapid exchange experiments special care was taken to avoid secondary phenomena like the "long-term" effect of high mucosal $[K^+]$ as described previously (Zeiske & Van Driessche, 1979), or the "toxic" effects of long-term exposure to Tl^+ (for details *see* Results and Discussion), by strictly limiting the mucosal exposition time to solutions containing a particular "K^+-like" cation to not more than 30 sec. This time limit allowed the full expression of the specific SCC features which were needed for the analysis of the current kinetics. The so-restricted method yielded fully reproducible results.

Fluctuation Analysis: Setup and Recording

A revised and further developed design of the previously described (Van Driessche & Zeiske, 1980*a, b*) setup for the SCC-fluctuation analysis has been presented recently (Wills, Zeiske & Van Driessche, 1982; Zeiske, Wills & Van Driessche, 1982). The data were processed in real-time by the use of two central processing units, one of which (Intel, SBC 80/30) did the data sampling while the other (Intel, SBC 86/12) calculated the power spectra. The final, averaged spectrum obtained under a particular experimental condition was stored on disk of a PDP 11/34 computer; there the spectra were fitted as described before (Van Driessche & Zeiske, 1980*b*) and plotted (*see* Figures) via a Hewlett-Packard 7221A plotter.

The long-term effect of Tl^+ did not much disturb the noise-data acquisition as long as the exposure time did not exceed three minutes. This was enough time to (*i*) change the solution in the special "high-seal" Ussing chamber for noise measurements (Van Driessche & Zeiske, 1980*a*), (*ii*) close off the noise setup in a Faraday cage, and (*iii*) start, after current stabilization, the data sampling to finally obtain 20 to 30 spectra to average.

Fluctuation Analysis: Theory and Spectral Fits

To evaluate the spectra the following model was used which describes (Van Driessche & Zeiske, 1980*a, b*; Zeiske & Van Driessche, 1981; Wills et al., 1982) the generation of Lorentzian-type power spectra from spontaneous current noise: An ionic channel switches randomly between one closed and one open, conducting, conformation.

$$\underset{⓪}{\text{open}} \underset{\alpha_{10}}{\overset{\alpha_{01}}{\rightleftharpoons}} \underset{①}{\text{closed}}.$$

The power spectrum $S_M(f)$ of the respective current fluctuations is a Lorentzian function (Eq. 1) describing the frequency (f) dependence of the noise power:

$$S_M(f) = S_o/(1+(f/f_c^{(1)})^2) \tag{1}$$

with S_o being the low-frequency plateau (*cf.* Van Driessche & Zeiske, 1980*a, b*) and the corner frequency $f_c^{(1)} = f(\text{at } S_o/2)$. $f_c^{(1)}$ is related to the rate constants (α_{01} and α_{10}) of the open-close reaction by

$$2\pi f_c^{(1)} = \alpha_{01} + \alpha_{10}. \tag{2}$$

In presence of the channel blocker Ba^{2+}, a three-state model (Van Driessche & Zeiske, 1980*b*)

$$\underset{②}{\text{blocked}} \underset{\alpha_{20}}{\overset{\alpha_{02}[Ba^{2+}]}{\rightleftharpoons}} \underset{⓪}{\text{open}} \underset{\alpha_{10}}{\overset{\alpha_{01}}{\rightleftharpoons}} \underset{①}{\text{closed}}$$

describes the generation of a double-Lorentzian power spectrum which is the sum $S_D(f)$ of two Lorentzian functions

$$S_D(f) = S_o^{(1)}/(1+(f/f_c^{(1)})^2) + S_o^{(2)}/(1+(f/f_c^{(2)})^2). \tag{3}$$

Our previous work (Van Driessche & Zeiske, 1980*b*) shows that the coupling between the two competing reactions will be minimal as long as $2\pi f_c^{(2)} < 50\%$ of $2\pi f_c^{(1)}$. Then the Ba^{2+}-channel reaction rate can be described (*see* Fig. 8) by

$$2\pi f_c^{(2)} = \alpha_{02}[Ba^{2+}] + \alpha_{20} \tag{4}$$

with the α's being rate constants. Thus the channel block by Ba^{2+} can be evaluated by considering it to be a pseudo first-order, two-state, reaction.

The spectral fits were done as described before (Van Driessche & Zeiske, 1980*b*) with

$$S(f) = K_b/f^{\alpha} + S_M(f)$$

or

$$S(f) = K_b/f^{\alpha} + S_D(f),$$

according to the observation of one, or two, Lorentzian components in the spectra, and a linear low-frequency background noise K_b/f^{α} (K_b, α being constants). Mean values are given ± SEM.

Solutions

In all experiments the serosal solution was frog Ringer's of the composition (in mM): 115 Na^+, 2.5 $KHCO_3$, 1 Ca^{2+} (pH 8.4). For rapid flow experiments serosal Cl^- was used throughout. For noise experiments with Tl^+, Cl^- was replaced by NO_3^-. The solution was bubbled with air except for the recording period of the current noise. The mucosal solutions contained the nitrates or chlorides of Tris (5 mM) and calcium (1 mM). The main mucosal salts containing the K^+-like cation were (115 mM), NH_4Cl, RbCl, KCl, KNO_3 and $TlNO_3$. We used NaCl or $NaNO_3$ (115 mM) containing 50 μM of the Na^+-channel blocker (Lindemann & Voûte, 1976) amiloride (a gift of Merck, Sharp and Dohme, Ltd.) as "inert" salt. To assess the K^+-Tl^+ interaction, NO_3^- was the anion present. Ba^{2+} was added as nitrate throughout. For the noise experiments a washing procedure as described previously (Van Driessche & Zeiske, 1980*b*) was performed.

Results

Apparent Permeability of the K^+ Channel for Monovalent Cations

SCC Measurements and Current Kinetics

The "apparent permeability" of the K^+ channel was estimated from comparison of short-circuit

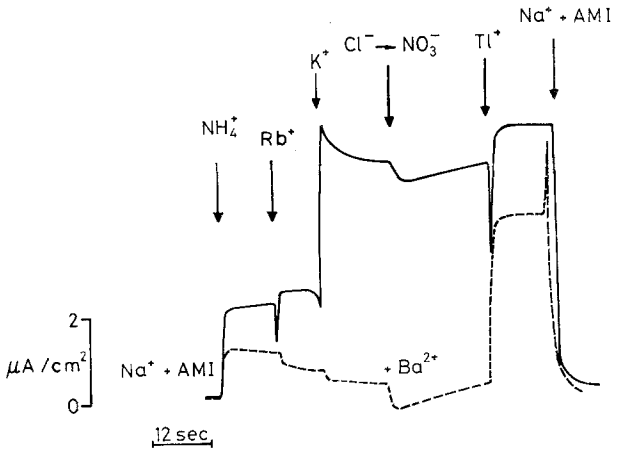

Fig. 1. Time course of the SCC when mucosal solutions are rapidly changed: Starting from a Na^+/amiloride (50 μM) Ringer's, the arrows indicate the moment when Na^+ was substituted, successively, by the desired K^+-like cations. Tl^+ was tested after Cl^- replacement by mucosal NO_3^-. Dashed line: Repetition of the experimental protocol in presence of 8 mM $Ba(NO_3)_2$ in the mucosal solutions. Serosal solution was NaCl-Ringer's throughout

current (SCC) obtained with various mucosal cations. With the main monovalent cation in the mucosal Ringer's at a concentration of 115 mM (NaCl Ringer's as serosal medium), a considerable positive SCC was recorded not only with K^+ but also with Rb^+, NH_4^+ and Tl^+ ions (Fig. 1): When the mucosal Na^+-Ringer's (containing amiloride to block Na^+ current) was successively replaced by NH_4^+, Rb^+ or K^+ Ringer's, respectively, the SCC rose instantaneously and reached maximal values, as in this experiment, generally of the order $NH_4^+ < Rb^+ < K^+$. In order to compare these ions with Tl^+, another "K^+-like" cation, mucosal Cl^- had to be substituted by NO_3^- thus preventing a possible precipitation of TlCl. This anion replacement resulted in a small but only transient SCC decrease. Exchanging then K^+ for Tl^+ produced – similar to the former cation substitutions – a rapid but quite large negative SCC transient. After this the SCC for Tl^+ leveled off at a current value somewhat larger than that for K^+ which was a frequent finding. Returning to Na^+-amiloride resulted in a quick drop of the SCC to zero, the value recorded before this experimental series. We note a special feature in Fig. 1, namely the steep and rapid negative SCC transients when one "K^+-like" ion is exchanged for another. A repetition of this experimental protocol in presence of a high dose of the K^+-channel blocker Ba^{2+} (Van Driessche & Zeiske, 1980*b*) in the mucosal solutions shows much reduced currents with the different cations, especially with K^+. The Ba^{2+} concentration used here is sufficient to totally suppress the K^+ current (Van Driessche & Zeiske, 1980*b*) and, as shown further below (*cf.* Fig. 6*a*), also the NH_4^+ and Rb^+ current. Although we see a different "nonspecific" SCC with NH_4^+, Rb^+ and K^+ in the presence of Ba^{2+} we may conclude that, besides K^+, also NH_4^+ and Rb^+ can pass the K^+ channel in the apical cell membrane. The fact that a good part of the Tl^+-dependent SCC can be suppressed by Ba^{2+} indicates the same to be true for Tl^+ ions.

The observed SCC changes are rapid and perfectly reversible which suggests that we observe the response of the apical skin border alone to the different cations. We might therefore, at this point, speculate that the magnitude of the Ba^{2+}-blockable SCC reflects the "permeability" of the apical K^+ channel for K^+-like cations, like NH_4^+ and Rb^+. The analogy to findings in excitable membranes is obvious (Hagiwara & Takahashi, 1974; Hille, 1975; Reuter & Stevens, 1980; Stanfield, Ashcroft & Plant, 1981). There, Tl^+ also permeates the K^+ channels, and it is even preferred over K^+, a conclusion which is not, however, directly suggested by the experiment in Fig. 1 as Ba^{2+} blocks only a part of the SCC in presence of Tl^+. At any rate the K^+-like cations do penetrate the frog skin's K^+ channel. When the passage of Li^+ and Na^+ through the Na^+ channel is blocked by amiloride these ions (of course not K^+-like) do not evoke a significant SCC. Similarly the K^+-like Cs^+ ion, usually not permeant through K^+ channels in excitable membranes (Hille, 1975) cannot pass the K^+ channel in frog skin but rather blocks the K^+ current (Zeiske & Van Driessche, 1979).

We also tested the organic ammonium derivates hydrazinium, hydroxylammonium, guanidinium, and larger molecules like tri- or tetramethylammonium for "permeability" (i.e. SCC). As observed with Cs^+ those compounds were not able to evoke any significant, nor Ba^{2+}-inhibitable SCC.

Contrary to the results with K^+ (Zeiske & Van Driessche, 1979), the specific, i.e. Ba^{2+}-blockable, currents with the other K^+-like permeant ions do not exhibit Michaelis-Menten kinetics and appear to saturate (if at all) at considerably higher ionic concentrations. This is demonstrated in Fig. 2*a*, where a comparison of the Rb^+-, NH_4^+- and K^+-currents, for the same preparation and ion concentrations up to 115 mM, reveals a clear current saturation with K^+ whereas, for Rb^+, this is only slightly indicated. For NH_4^+ the upward bent dose-response curve does not point towards any saturation at all within the investigated concentration

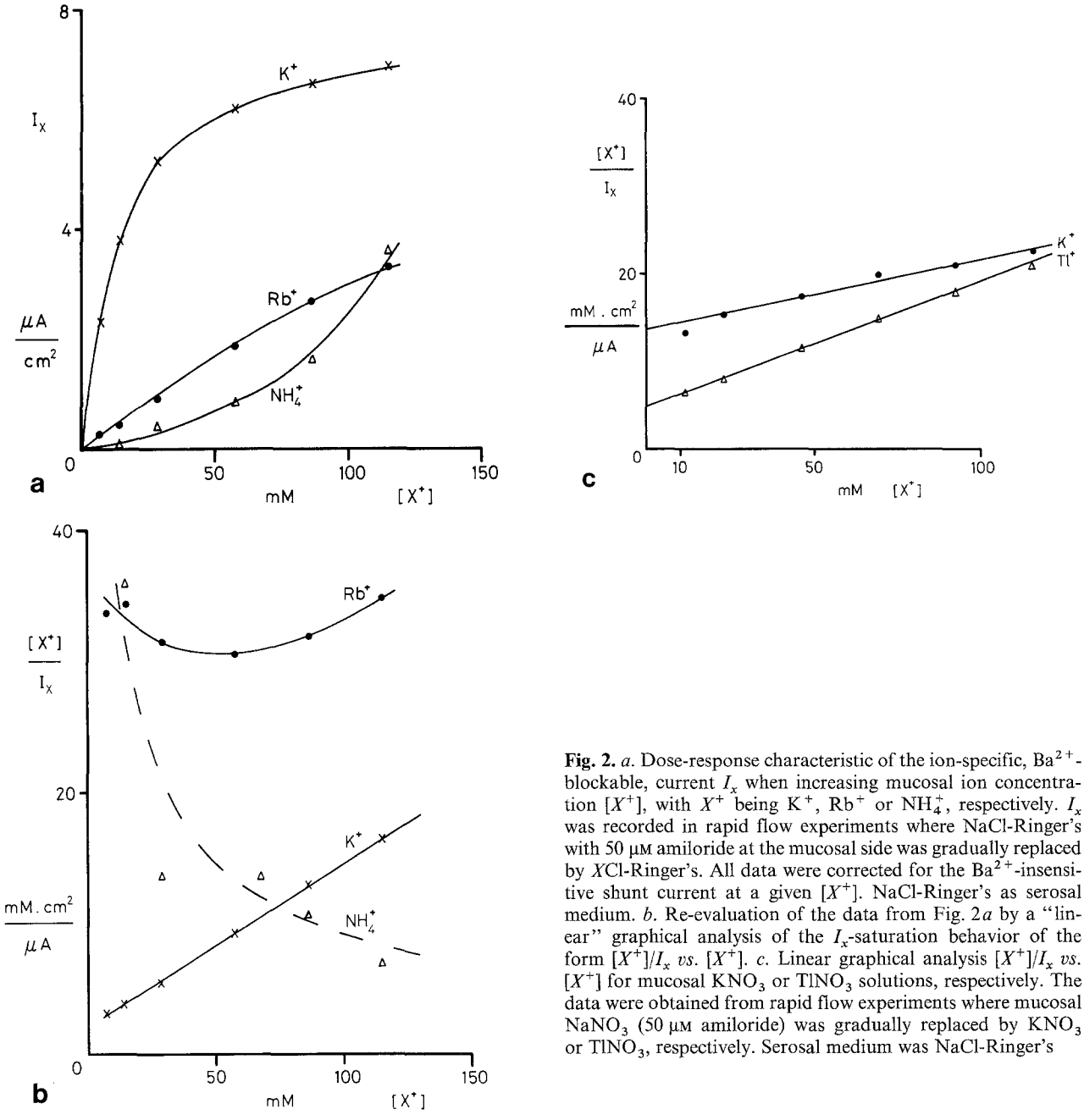

Fig. 2. *a.* Dose-response characteristic of the ion-specific, Ba^{2+}-blockable, current I_x when increasing mucosal ion concentration $[X^+]$, with X^+ being K^+, Rb^+ or NH_4^+, respectively. I_x was recorded in rapid flow experiments where NaCl-Ringer's with 50 μM amiloride at the mucosal side was gradually replaced by *X*Cl-Ringer's. All data were corrected for the Ba^{2+}-insensitive shunt current at a given $[X^+]$. NaCl-Ringer's as serosal medium. *b.* Re-evaluation of the data from Fig. 2*a* by a "linear" graphical analysis of the I_x-saturation behavior of the form $[X^+]/I_x$ *vs.* $[X^+]$. *c.* Linear graphical analysis $[X^+]/I_x$ *vs.* $[X^+]$ for mucosal KNO_3 or $TlNO_3$ solutions, respectively. The data were obtained from rapid flow experiments where mucosal $NaNO_3$ (50 μM amiloride) was gradually replaced by KNO_3 or $TlNO_3$, respectively. Serosal medium was NaCl-Ringer's

range; it rather resembles the initial part of S-shaped functions as seen for cooperative channel-saturation. From this Figure we may nevertheless estimate orders of the apparent half-maximal ion concentration (K_M), $K^+ < Rb^+ < NH_4^+$, and the saturation level of the current, $Rb^+ < K^+ < (?)NH_4^+$. Certainly, these orders are bound to the assumption of simple (Michaelis-Menten) saturation for Rb^+- and K^+ current, and an S-shaped dose-response relationship for NH_4^+ ions.

As was suggested by Lineweaver and Burk (*see* Westley, 1969) and already described for K^+ in previous publications (Zeiske & Van Driessche, 1979; Van Driessche & Zeiske, 1980*b*), a linear relation between the ratio of [ion concentration]/[specific current] versus concentration indicates Michaelis-Menten saturation. Figure 2*b* shows a re-evaluation of the data from Fig. 2*a* and demonstrates that only for K^+ the current behaves in a simple way indicating an interaction of the saturating channel site with one K^+ ion only. Clearly, for both Rb^+ and NH_4^+ this postulate does not hold. The apparent minimum for Rb^+, but even more the continuous decrease of the curve for

NH_4^+ with higher ion concentrations rather suggest that the ion-specific currents (with NH_4^+ and Rb^+) are subproportional to the ones expected for Michaelis-Menten behavior. Thus the initial linearity for Rb^+, in Fig. 2*a*, is only apparent and even close inspection might not have revealed the difference between K^+ and Rb^+ in this type of a dose-response graph.

The evaluation of the Tl^+-specific current seems problematic considering (*i*) the impossibility to determine a nonspecific Tl^+ shunt current with reasonable Ba^{2+} concentrations, and (*ii*) the unavoidable and irreversible long-term decrease of the Tl^+ current (*see below*). When a current analysis as described above is performed for the SCC in presence of Tl^+ (Fig. 2*c*) the graph shows linearity (despite the Tl^+—SCC could not be corrected for a nonspecific component) thus indicating, as for K^+, (*i*) a relatively negligible shunt path for Tl^+ as compared to the one for Rb^+ or NH_4^+ (*cf.* Fig. 1), and (*ii*) apparently simple saturation kinetics of the Tl^+ current through the apical K^+ channel.[1]

Dual Interaction of Tl^+ with the K^+ Channel

From most of the rapid ion substitution experiments (as in Fig. 1) it seems that the SCC in presence of Tl^+ tends to surmount that obtained with K^+. So one could expect simply an increase in current when small amounts of Tl^+ are added to the K^+ Ringer's. Surprisingly, the addition of low (here 8 mM) Tl^+ concentrations rather depresses the SCC (Fig. 3*a*). When now K^+, but not Tl^+, is replaced by Tris, the SCC remains positive as expected for solutions with Tl^+ only (*cf. also* Fig. 1). Since this small current obtained with a small concentration of Tl^+ (absence of K^+) can be totally blocked by 10 mM Ba^{2+} (*not shown*) we must conclude that the overall K^+-channel "permeability" can be depressed by the presence of a second, though permeant ionic species.

[1] The half-maximal concentration (negative abscissa intercept) is much larger here than in the experiment of Fig. 2*a*. One reason for this discrepancy could be the use of nitrate as ambient anion in this type of experiment, though the K^+ current does – at least for large $[K^+]$ – not seem to depend much on this anion (*cf.* Fig. 1). On the other hand the long-term effect of exposition to high $[K^+]$ (Zeiske & Van Driessche, 1979) could be responsible for the large scatter in half-maximal current. Though we limited the exposure time in order to avoid this effect, this particular skin could have been very sensitive. A survey of our data indicates that there might be a relationship between skin sensitivity to the long-term effect, and the time of the year (winter animals being more affected). At any rate, the result featured in Fig. 2*c* is the finding of linearity and thus Michaelis-Menten kinetics for both K^+ and Tl^+ current.

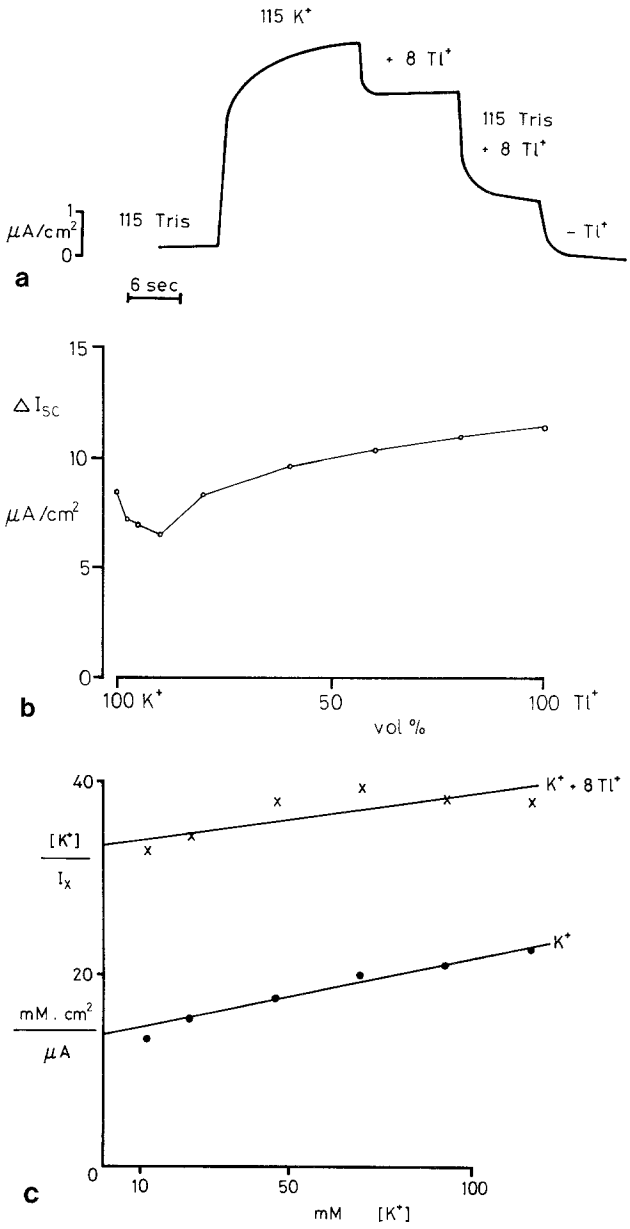

Fig. 3. *a.* Rapid flow exchange of mucosal Tris-NO_3 Ringer's for K^+ and Tl^+ (NO_3^-) as indicated. Time course of the SCC. Serosa: NaCl-Ringer's. *b.* Mole-fraction relationship of the SCC when mucosal KNO_3 is successively replaced by $TlNO_3$. ΔI_{sc} indicates the current change when mucosal $NaNO_3$/50 μM amiloride was replaced by the respective K^+/Tl^+ mixture. Serosa: NaCl-Ringer's. *c.* Linear graphical analysis $[K^+]/I_x$ ($x = K^+$ or $K^+ + Tl^+$, respectively) *vs.* $[K^+]$ in absence, and presence of, mucosal Tl^+ (8 mM). The data were obtained from rapid flow experiments as described for Fig. 2*c*

A similar $Tl^+ - K^+$ interaction has already been described for other K^+ channels (Landowne, 1975; Hagiwara, Miyazaki, Krasne & Ciani, 1977) as well as the gramicidin channel (Neher, 1975), and, as appropriate representation of this interaction, the so-called "mole fraction" relationship has

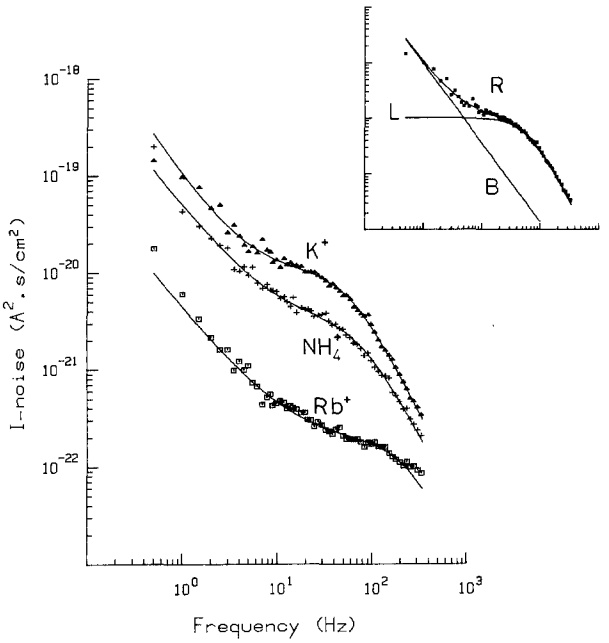

Fig. 4. Power spectra and fits (lines) obtained with either mucosal KCl (▲)-, NH_4Cl (+)- or RbCl (□)-Ringer's, respectively. For details *see* text. The inset shows, for K^+, how the linear background (*B*) and the Lorentzian component (*L*) superimpose to the resultant fit (*R*)

been used (Fig. 3*b*): Here, the SCC is displayed as a function of the molar cation-concentration ratio expressed in vol % for mixtures of Tl^+/K^+ Ringer's. At low $[Tl^+]/[K^+]$ this curve shows a minimum. We find, in most skins, a SCC with 100% Tl^+ equal or larger than compared to 100% K^+. Sometimes Tl^+ currents were smaller than K^+ currents, however by never more than 20 percent of the K^+ current. For the latter finding, a "long-term" effect observed only with high $[Tl^+]$ solutions could be responsible: Beyond 30 sec exposure to high $[Tl^+]$, the Tl^+-generated SCC starts to decrease irreversibly and almost disappears within the next 30 min. A corresponding decrease in overall-cation permeability has been observed (*unpublished findings*) which suggests an only secondary, apparently lower, skin permeability for Tl^+ than for K^+, in a part of the experiments. Another source of error could be individual differences in nonspecific skin permeabilities (*see* Fig. 1) as a complete blockage cannot be obtained with reasonable (<20 mM) Ba^{2+} concentrations. Nevertheless we feel, also in analogy to findings for K^+ channels in other membranes (Hille, 1975; Hagiwara et al., 1977; Reuter & Stevens, 1980; Stanfield et al., 1981), that the Tl^+ current through the apical K^+ channel is at least comparable in magnitude to the specific K^+ current. We shall further discuss this point below.

In order to study the nature of the K^+-current inhibition by low $[Tl^+]$ we recorded dose-response curves of the SCC with mucosal K^+, in absence and presence of 8 mM Tl^+. In Fig. 3*c*, a linear analysis of the saturating K^+ current is done as described before (Zeiske & Van Driessche, 1979) by a plot of $[K^+]/I_x$ *vs.* $[K^+]$. Here, I_x indicates the Ba^{2+}-corrected SCC in presence of either K^+ alone, or in presence of 8 mM Tl^+. In presence of Tl^+ the practically linear control curve is shifted upwards. The deviations of the upper data points from linearity (fit by eye) are apparently small. They may partly arise from the current dependence on the actual $[K^+]/[Tl^+]$ ratio (*cf.* Fig. 3*b*), but also from scatter in the very small SCC. Both lines are almost parallel; the small apparent decrease in slope of the upper line could well originate from appropriate multi-site interactions in the Tl^+/K^+ mixtures. Nevertheless we feel that we might interpret our findings to indicate a Tl^+ block in competition with K^+ ions for a common site of interaction with the K^+ channel. In presence of the "blocker," the maximal K^+ current (inverse slope) remains roughly constant but the Michaelis constant of the K^+-current saturation increases (higher abscissa intercept). Thus, with the above reservations, and at low $[Tl^+]/[K^+]$ ratios, Tl^+ behaves like the impermeant K^+-channel blockers Cs^+ (Zeiske & Van Driessche, 1979) and Ba^{2+} (Van Driessche & Zeiske, 1980*b*).

Noise Measurements

In several publications we described the microscopic properties of the apical K^+ channel as obtained from fluctuation analysis of the K^+ current (Van Driessche & Zeiske, 1980*a, b*; Zeiske & Van Driessche, 1981). A Lorentzian component in the power spectrum of the current noise was interpreted in terms of a K^+ channel switching randomly between a nonconducting and one conducting state. With a K^+ channel also being permeable for NH_4^+, Rb^+ and Tl^+, we may consequently expect a Lorentzian component in the noise of the current generated by high mucosal concentrations of these ions. Figure 4 depicts power spectra for the case where the same skin was exposed to either NH_4^+, Rb^+, or K^+ Ringer's on the mucosal side. We clearly see that a Lorentzian component is present not only with K^+ but also with NH_4^+. The inset demonstrates, for K^+, the Lorentzian component (*L*) and the linear background (*B*) whose sum is the resultant fit (*R*) of the spectral data. In presence of Rb^+ the spectral intensity is much lower

but a Lorentzian function is indicated by a shoulder in the middle frequency range. The spectral fits yield values for the Lorentzian plateaus ($S_o \times 10^{21}$ in A^2 sec/cm^2), for K^+: 10.0; for NH_4^+: 3.0; for Rb^+: 0.18. The resulting corner frequencies f_c (f where $S = S_o/2$) are (in Hz) 58.6 for K^+, 76.1 for NH_4^+ and 230 for Rb^+. The experiment shown here is one out of only two where we could see a Rb^+ Lorentzian besides the always visible Lorentzians with K^+ or NH_4^+. Obviously the Rb^+ Lorentzian was not clearly above the background noise (in this case linear component + amplifier noise) but indicated in many experiments, by a flatter middle part in the spectrum. For a number of skins ($n = 15$) the average value of Lorentzian plateau was $(8.5 \pm 0.5) \times 10^{-21}$ A^2 sec/cm^2 for K^+, and $(3.8 \pm 0.3) \times 10^{-21}$ A^2 sec/cm^2 for NH_4^+. The respective mean f_c was (55.9 ± 3.5) Hz for K^+, and (65.7 ± 3.1) Hz for NH_4^+. In the two successful fits of Rb^+ spectra we obtained the plateau values 1.8×10^{-22} and 6.4×10^{-22} A^2 sec/cm^2, with the respective f_c of 230 and 161 Hz.

At this point we may conclude that, besides K^+, also Rb^+ and NH_4^+ can pass the fluctuating K^+ channel. However, while some differences in the Lorentzian plateau values can be expected when different intrinsic permeabilities result in different specific currents (Fig. 1), the change in corner frequency is surprising. It means that a particular ionic species can influence the channel gating. This gating proceeds with much higher frequencies in presence of Rb^+, but is also constantly found to be faster with NH_4^+ as compared to K^+. Apart from other factors the finding that $f_c(Rb^+)$ is considerably larger than $f_c(NH_4^+, K^+)$ might be responsible (*cf.* Van Driessche & Zeiske, 1980*b*) for the comparatively small $S_o(Rb^+)$ though the ion-specific currents do not differ very much (Fig. 1).

When mucosal K^+ is replaced by Tl^+ Ringer's (NO_3^-!), and when the exposition time to Tl^+ is brief (< 3 min), a Tl^+ Lorentzian noise can be observed (Fig. 5*a*) whose plateau value is often of a magnitude comparable to that for K^+; however, f_c is always clearly increased (mean: (89.3 ± 8.1) sec^{-1}, $n = 8$). In a few cases (*see* Fig. 5*a*) we found $S_o(Tl^+) > S_o(K^+)$. But in many skins the plateau of the Tl^+ Lorentzian was considerably smaller than of the K^+ Lorentzian. As was already mentioned the Tl^+ current tends to decrease irreversibly at longer (> 1 min) exposition times; also other ionic currents (K^+ or Na^+) suffer from this toxic effect and are irreversibly depressed after the skin has been in contact with Tl^+ (*unpublished observations*). In Fig. 5*b* it can be seen that long-term exposure to Tl^+-Ringer's completely abolishes the

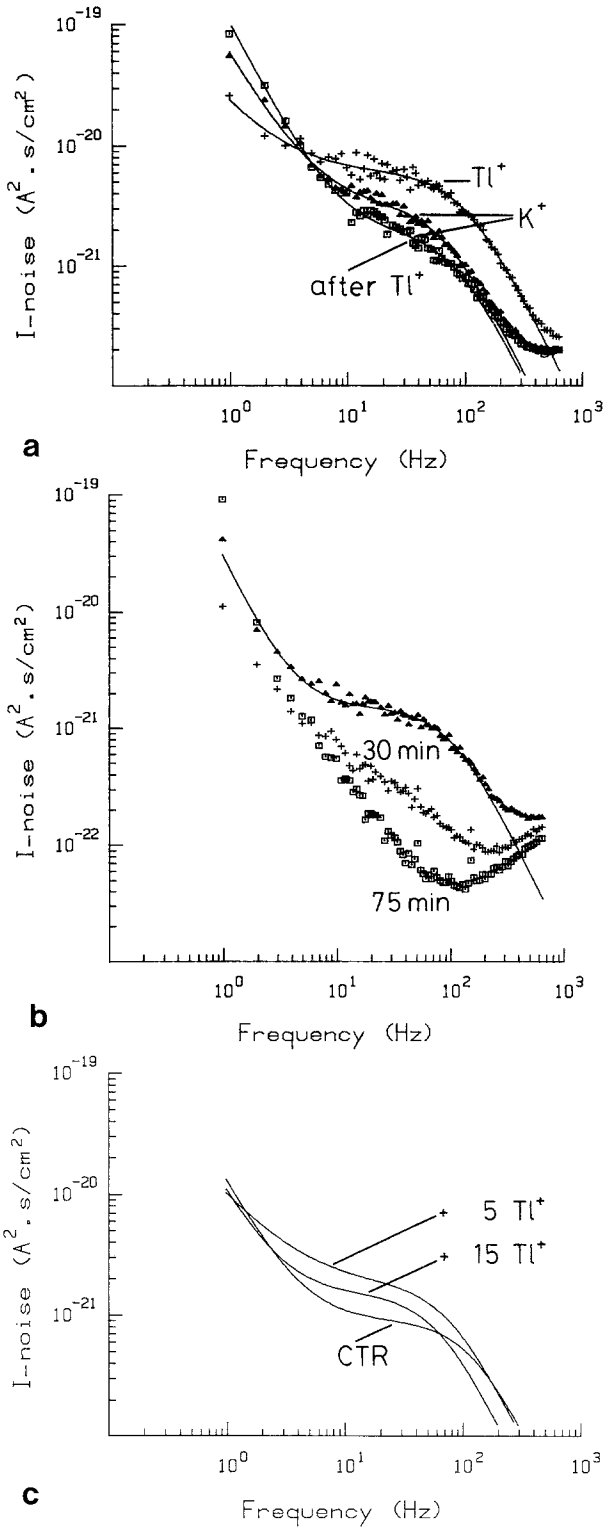

Fig. 5. *a.* Comparison of power spectra and spectral fits for the case of mucosal KNO_3 before (▲), and after (□) Tl^+ was tested, and with mucosal $TlNO_3$-Ringer's alone (+). *b.* Time course of the abolishment of the Tl^+-dependent Lorentzian noise: begin (▲), 30 min (+) and 75 min (□) of exposure to mucosal $TlNO_3$-Ringer's. *c.* Power spectra obtained with KNO_3-Ringer's, KNO_3 plus 5 or 15 mM $TlNO_3$ as mucosal medium. For clarity, spectral data were omitted; only fits are shown

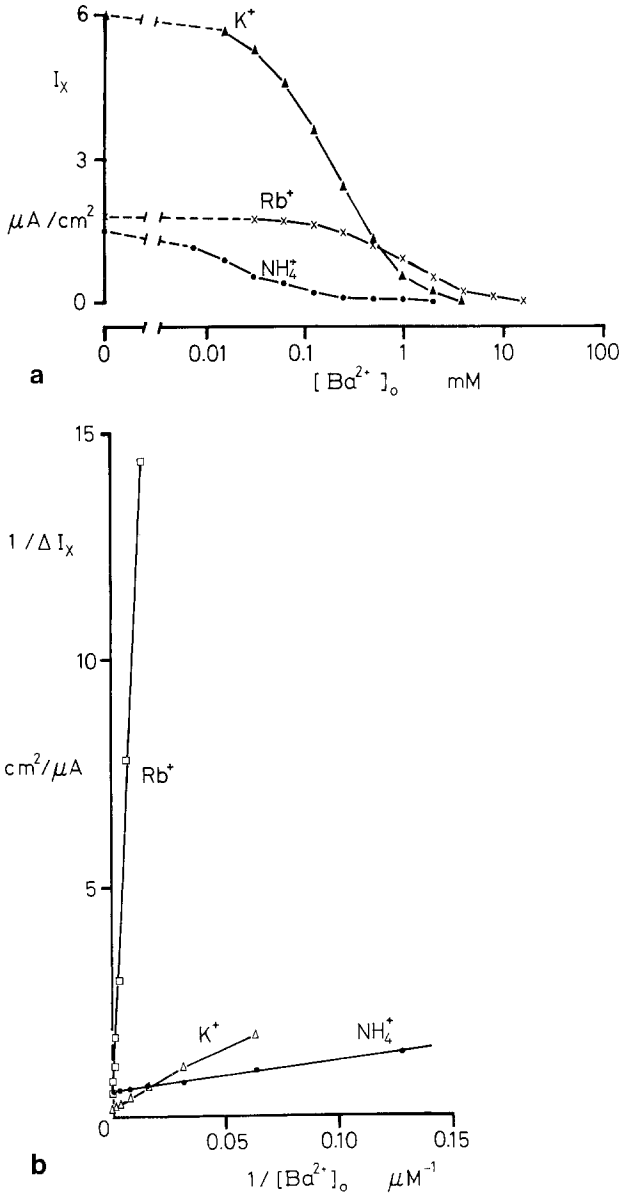

Fig. 6. *a*. Dose-dependent depression of the ion-specific current, I_x, by increasing concentrations of mucosal Ba^{2+}, $[Ba^{2+}]_o$. Mucosal solutions were KCl (▲)-, RbCl (×)- or NH_4Cl (●)-Ringer's. The experimental data were obtained by rapid flow measurements. Serosa: NaCl-Ringer's. *b*. Double-reciprocal plot of the data from Fig. 6*a* for K^+ (△), Rb^+ (□) and NH_4^+ (●). The lines were drawn by eye

Tl^+-dependent Lorentzian noise. This effect might then also explain why – after Tl^+ – the K^+ Lorentzian is clearly reduced as compared to before Tl^+ (Fig. 5*a*).

Recalling the inhibitory effect of low $[Tl^+]$ on the K^+ current (Fig. 3*a, b*) we expect a depressed K^+ noise in presence of low $[Tl^+]$. However, the addition of 5 mM Tl^+ to K^+ Ringer's causes a considerable shift of the K^+-Lorentzian parameters: lower f_c, and a higher S_o (Fig. 5*c*). Such shifts have already been described (Zeiske & Van Driessche, 1981) and occur when surface-charge screening cations are present in the mucosal K^+-Ringer's, thereby slowing down the K^+-channel gating by increasing the mean closed time. We may interpret the effect of 5 mM Tl^+ on the K^+ noise analogously.

With respect to this new situation (supposedly having Tl^+-modified K^+-channel gating) we eventually obtained the expected decrease (Fig. 5*c*) in K^+-Lorentzian noise with a further rise of the Tl^+ concentration to 15 mM, which is close to the maximal inhibitory effect of Tl^+ ions on K^+ current (Fig. 3*b*). Yet, the plateau in presence of Tl^+ is still larger than without, but f_c does not change any more. Thus, apart from the modifying influence of low $[Tl^+]$ on channel kinetics in the presence of high $[K^+]$, the dual behavior of Tl^+ ions – K^+ inhibitory at low but permeant at high concentration – can also be followed by noise analysis. Like NH_4^+ and Rb^+ ions, Tl^+ also causes an increase in channel-gating frequency, when tested at Ringer's concentration.

The Channel Block by Mucosal Ba^{2+}

Analysis of Steady-State Kinetics from SCC

The dose-response relation for the inhibitory action of Ba^{2+} is shown, for one skin, in Fig. 6*a*: Increasing amounts of Ba^{2+} reduce the currents generated by mucosal NH_4^+, Rb^+ or K^+ Ringer's, respectively, in an S-shaped manner when plotted in a semi-logarithmic diagram (the plot only shows ion-specific, Ba^{2+}-blockable currents). Michaelis-Menten kinetics of the dose-response curves are apparent but the Ba^{2+} dose required for a half-maximal block strongly depends on the permeant ion under investigation. A re-evaluation (Fig. 6*b*) of the data in Fig. 6*a* by a double-reciprocal plot shows straight lines, thus evidence for simple saturation kinetics of the Ba^{2+}-blocking reaction. Clearly, Ba^{2+} blocks most efficiently the NH_4^+ current, less the K^+ current and least the Rb^+ current. The Ba^{2+} concentrations for a half-maximal dose, K_{Ba}, are 13 μM for NH_4^+, 160 μM for K^+, and 1 mM for Rb^+, in this experiment.

Previously (Van Driessche & Zeiske, 1980*b*) we showed the competition between K^+ and Ba^{2+} by graphical analysis. In a plot such as in Fig. 3*c* a competitive inhibition would be indicated by a parallel upward shift of the straight line for K^+. As a graphical analysis yields an easily interpretable result only for systems with simple saturation behavior (K^+) its application to evaluate the type

of Rb^+- and NH_4^+-current inhibition by Ba^{2+} therefore seems futile. For the simpler interaction of Tl^+ with the K^+ channel (absence of K^+!) a graphical analysis to characterize the type of Tl^+/Ba^{2+} interaction may seem useful. However, no parallel upwards shift of the straight control line (as in Fig. 3*c*) was obtained but rather a change in slope and ordinate intercept, as well as some minor curvature (*not shown*). This indicates that the presence of Ba^{2+} somehow modifies the ionic channel's handling of Tl^+. Inspection of the estimated parameters from the current analysis suggests that the parameter K_{Ba} is not inversely proportional to the reciprocal K_M value of the permeant ion as a simple competition model would predict (Van Driessche & Zeiske, 1980*b*). Then, for NH_4^+ and Rb^+, one would expect a K_{Ba} of the same order of magnitude but not a 100-fold difference as experimentally observed.

Rate Constants for the Ba^{2+} Block from the Analysis of Ba^{2+}-Induced Lorentzian Noise

We have reported earlier (Van Driessche & Zeiske, 1980*b*) that Ba^{2+} induces a second, low-frequency, Lorentzian noise in the power spectrum. The Ba^{2+}-induced noise was interpreted to originate from the random blockage of the spontaneously open K^+ channel by Ba^{2+} ions, thus resulting in an additional on- and off-switching of the channel conductance, this time dependent on Ba^{2+}. Since Ba^{2+} also depresses NH_4^+-, Rb^+- and the Tl^+-current one might expect a Ba^{2+}-induced low-frequency Lorentzian in presence of these permeant ions, too.

Figure 7*a* presents power spectra recorded with either mucosal NH_4^+, Rb^+ or K^+ Ringer's, all in the presence of 40 μM Ba^{2+}. In the case of K^+ we can observe the shoulder from the spontaneous Lorentzian noise at higher frequencies (corner frequency $f_c^{(1)}$), whereas the Ba^{2+}-induced component is much more intense and has a low corner frequency ($f_c^{(2)}$). The f_c of the Lorentzian in presence of NH_4^+ is much smaller than expected if it were spontaneous NH_4^+ noise (*cf.* Fig. 4). Contrary to the extremely rare observation of a spontaneous Rb^+-relaxation noise, a Ba^{2+}-induced component could always be obtained with Rb^+ as main mucosal cation (Fig. 7*a*). Here also, the $f_c^{(2)}$ is much smaller than $f_c^{(1)}$ of the spontaneous Lorentzian noise with Rb^+ alone (*cf.* Fig. 4). The plateaus of the Ba^{2+}-induced Lorentzians are in the order $K^+ \gg Rb^+ \approx NH_4^+$. More important, for a particular Ba^{2+} concentration, the f_c values of the induced noise were usually different, indicating different

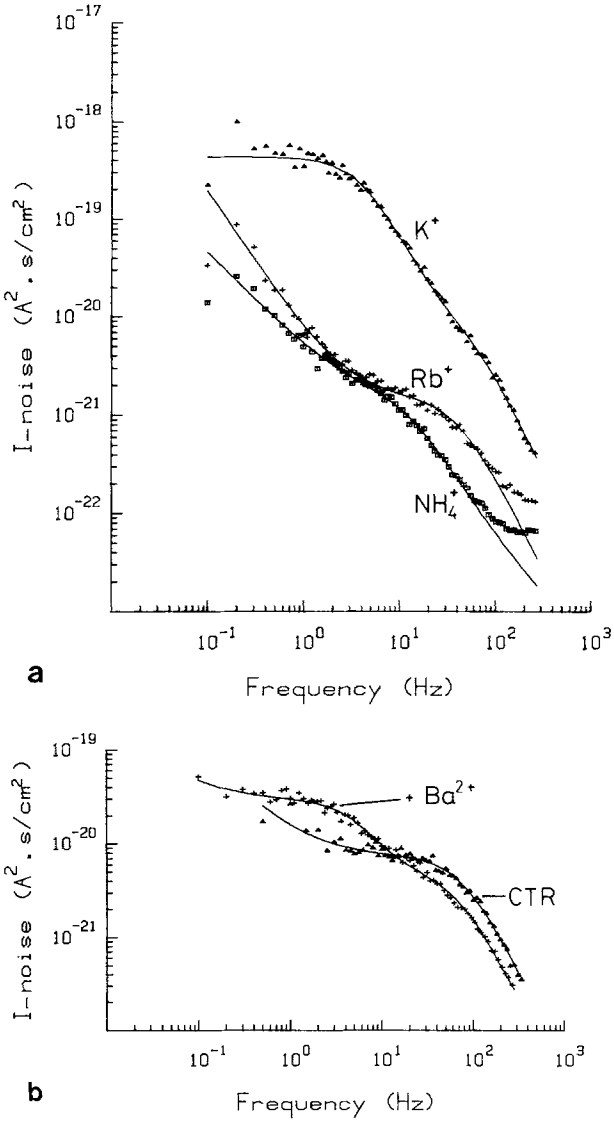

Fig. 7. *a.* Power spectra obtained with 40 μM $[Ba^{2+}]_o$ in either KCl (▲)-, RbCl (+)- or NH_4Cl (■)-Ringer's as mucosal medium. *b.* Power spectra obtained with mucosal $TlNO_3$-Ringer's alone (▲), and in presence (+) of 200 μM $[Ba^{2+}]_o$

blocking kinetics when different permeant ions are present. For the case of Tl^+, a Ba^{2+}-induced Lorentzian was found only rarely. Such an experiment is shown in Fig. 7*b* with spectra in absence and presence of Ba^{2+}-induced Lorentzian which also has a much smaller f_c than the spontaneous channel noise.

As seen for the K^+/Ba^{2+} interaction (Van Driessche & Zeiske, 1980*b*), increased $[Ba^{2+}]$ shifts the Ba^{2+}-induced Lorentzian to higher corner frequencies (but lower plateau values), also for the case of NH_4^+ and Rb^+ (Fig. 8). For K^+ it has been established (Van Driessche & Zeiske, 1980*b*) that the spontaneous K^+-channel fluctuations are characterized by a probability of almost 1 to find

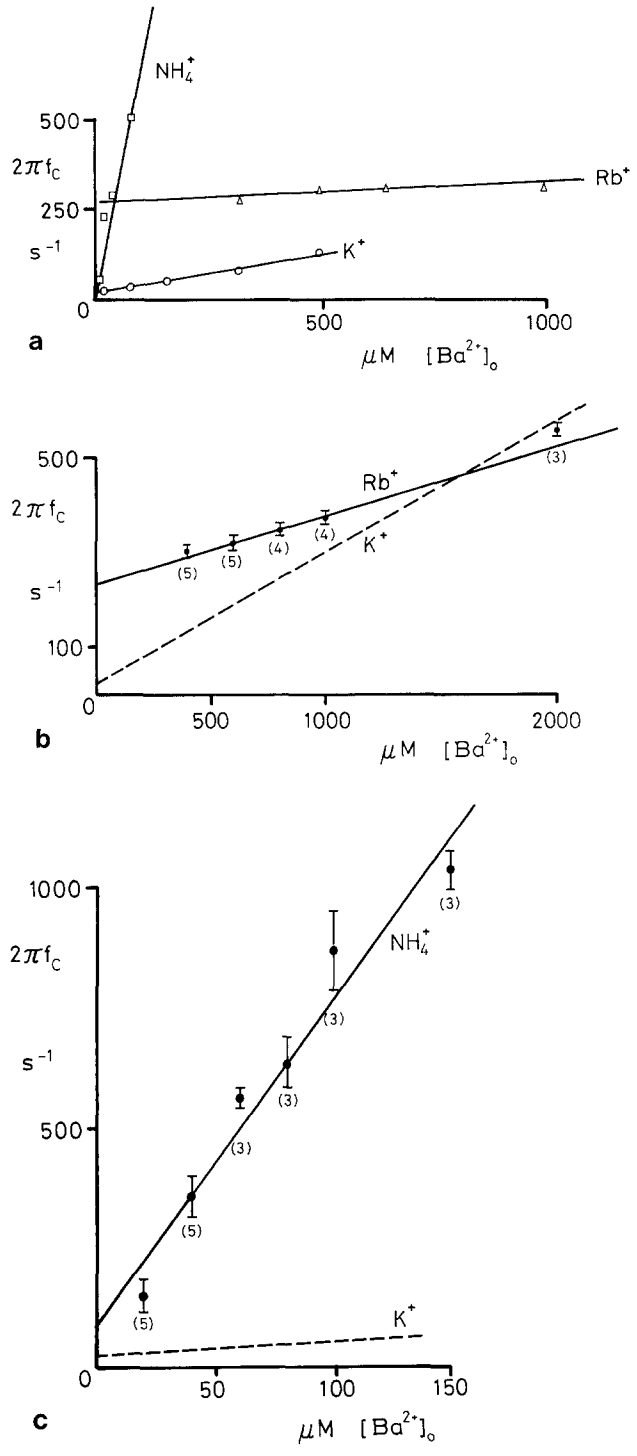

Fig. 8. *a.* Linear relationship between the corner frequency of the Ba^{2+}-induced Lorentzian noise and the mucosal Ba^{2+} concentration, with either KCl (o)-, RbCl (△)- or NH_4Cl (□)-Ringer's as mucosal medium. The Ba^{2+}-concentration range was limited by the necessity to discriminate the Ba^{2+}-dependent relaxation noise from the background. *b, c.* Pooled data (number of skins in brackets; ± SEM) for the relation $2\pi f_c$ *vs.* $[Ba^{2+}]_o$, from the Ba^{2+}-induced Lorentzian noise, in presence of mucosal RbCl (*b*) or NH_4Cl (*c*) Ringer's and in comparison to mucosal KCl (stippled; from Van Driessche & Zeiske, 1980*b*). The lines are obtained by linear regression

a K^+ channel in its open conformation. In this case the channel reaction with Ba^{2+} could be regarded as a quasi two-state mechanism thereby neglecting the influence of the spontaneous channel fluctuations as long as the Ba^{2+} concentration is small (condition: $f_c^{(2)} < 1/2f_c^{(1)}$ – *see* Materials and Methods). Then, the finding of a linear relation between $2\pi f_c$ (of Ba^{2+}-induced Lorentzian) and $[Ba^{2+}]$ expresses the pseudo first-order kinetics of the Ba^{2+}-channel interaction: In such a plot the slope is equal to the rate constant α_{02} for the association of Ba^{2+} at its receptor (channel blocked) while the ordinate intercept is the dissociation rate constant, α_{20}. The apparent Michaelis constant for the Ba^{2+} block is then $K_{Ba} = \alpha_{20}/\alpha_{02}$. Figure 8*a* suggests (linearity!) that also the Ba^{2+} block of NH_4^+ and Rb^+ current is conformable with the quasi "two-state" model of channel block. However, the precondition for the applicability of a two-state description for the Ba^{2+} block obviously is not met with NH_4^+ as, already at very small $[Ba^{2+}]$, the corner frequency of the Ba^{2+}-induced Lorentzian surpasses that observed for the spontaneous channel noise. In Fig. 8*a*, the data obtained from the same skin show that the $f_c^{(2)}$-dependence on $[Ba^{2+}]$ is much more pronounced with NH_4^+ as the permeant ion, much less with K^+, and least with Rb. We pooled data from various skins, averaged them and analyzed the $2\pi f_c$-$[Ba^{2+}]$ relationship by linear regression (Fig. 8*b, c*). When compared with the $2\pi f_c$-$[Ba^{2+}]$ relation for K^+ (dashed line; from Van Driessche & Zeiske, 1980*b*) it becomes apparent that, on the basis of the quasi two-state model the considerable differences in K_{Ba} (*cf.* Fig. 6) would be due to differences in both association (slope) and dissociation (ordinate intercept) rate constants. The K_{Ba} from these plots compare favorably with the ones obtained from Fig. 6*a*. The rate parameters α_{02} and α_{20} as obtained from the regressions lines do, at first sight, not suggest a systematic correlation between on- and off-rate for the Ba^{2+} block in presence of a particular permeant ion.

Due to the specific problems with Tl^+ the available data are not sufficient for a rigorous analysis of the Ba^{2+}-induced Tl^+ noise. However, a preliminary conclusion may be drawn from the observation (*unpublished*) that the $f_c^{(2)}$ for this case seems not to shift with $[Ba^{2+}]$: This means that the association rate of Ba^{2+} at its receptor should be relatively small in presence of Tl^+ while the measured $f_c^{(2)}$ (5 sec^{-1}) would roughly equal α_{20}, in the two-state model. These findings agree with the observed, comparatively weak effect of Ba^{2+} on the Tl^+ current (*cf.* Fig. 1) and support the idea of

a common site for the inhibitory action of Tl^+ and Ba^{2+} on the K^+ current.

Discussion

Besides K^+, also NH_4^+, Rb^+ and Tl^+ pass the fluctuating K^+ channel in the apical membrane with different apparent "permeabilities." The ionic currents seem to obey Michaelis-Menten kinetics with K^+ and Tl^+ but indicate cooperative effects in presence of NH_4^+ and Rb^+; the estimated current saturation seems to occur at much lower concentrations with K^+ and Tl^+ than with NH_4^+ and Rb^+. The noise in the currents generated by these ions displays a relaxation component in all cases; however, the respective corner frequencies differ clearly.

Mixtures of K^+ and Tl^+ reveal the dependence of the "permeability" of the channel for one ion on the presence, as well as concentration of the other: recordings of the macrosopic current and power spectra both reflect this interaction. At low concentrations Tl^+ behaves like a competitive inhibitor of the K^+ current.

As for K^+, the passage of NH_4^+, Rb^+ and Tl^+ is also blocked by Ba^{2+}. The mode of inhibition could not clearly be established. The efficiency of the Ba^{2+} block strongly depends on the nature, and not simply on the channel affinity of the permeant ion which is present together with Ba^{2+}. The channel block by Ba^{2+} induces additional current fluctuations of a Lorentzian type (corner frequency $f_c^{(2)}$), for all permeant ions. From those, the analysis of the linear $f_c^{(2)}$-$[Ba^{2+}]$ relation permits, in the frame of a common kinetic model, the estimation of the rate constants for the association and dissociation reactions of Ba^{2+} at its receptor site. Both parameters depend on the nature of the specific permeant ion, and their considerable differences explain the variable efficacy of the Ba^{2+} inhibition of the ionic currents.

Problems Arising in the Evaluation and Interpretation of the Experimental Data

The general problems in recording and interpreting Lorentzian (and background) noise from epithelia have been discussed in length before (Van Driessche & Zeiske, 1980*a*, *b*; Zeiske & Van Driessche, 1981; Wills et al., 1982; Zeiske, Wills & Van Driessche, 1982) which will not be repeated here. Our major concern has been to avoid falsification of the data by the long-term exposure effects which were observed in presence of Tl^+ ions. For these experiments the usual short-term exposure for steady-state kinetic analysis was probably preventing problems due to the irreversible effects of Tl^+ ions. Intoxication by Tl^+ has been reported for almost all types of cells and mechanisms like K^+-channel blockage (Hagiwara et al., 1977) or uptake of Tl^+ via the K^+ site at the (Na^+/K^+)-ATPase molecule (Britten & Blank, 1968; Landowne, 1975) could lead to damage in cell function, e.g. by reacting with SH groups of proteins. For noise recordings with longer exposure periods (>3 min), the toxic Tl^+ effect became problematic as has been demonstrated in Fig. 5. Due to these complications which are likely to have caused the poor yield of Tl^+-noise data, a less thorough and rather qualitative analysis only was possible.

Transport Properties of the Fluctuating K^+ Channel

Analysis of the Macroscopic Currents

Evidence for Multi-Site Single Filing. Recently it has been shown for permeation pathways across cell membranes (Hille, 1975; Hagiwara et al., 1977; Sandblom, Eisenmann & Neher, 1977; Hille & Schwarz, 1978; Urban & Hladky, 1979; Finkelstein & Andersen, 1981) and even paracellular junctional complexes (Fromm & Schultz, 1981; Salas & Moreno, 1982) that ionic diffusion cannot always be described by the "independence principle" (Goldman, 1943; Hodgkin & Katz, 1949) but rather by "single-file diffusion" via multi-site channels. In our preparation, we found several hints for single-filing in the cumulating occurrence of phenomena such as: saturation of ionic currents including cooperativity; competitive inhibitory occlusion by impermeant (Cs^+, Ba^{2+}) or even permeant ions (Tl^+ *vs.* K^+); a strong concentration- and ion-dependent channel "permeability" as suggested by complex current kinetics with one permeant ion present (Rb^+, NH_4^+). The anomalous mole-fraction relationship in presence of two permeant ionic species certainly is the most convincing evidence (Hille & Schwarz, 1978). This is also expressed in the fast current-transients during ionic substitutions. Furthermore, we observed a strong influence of the permeant ions on the K^+-channel gating rate (f_c), and, finally, a clear dependence of the Ba^{2+}-blocking action on the species of the permeant ion, expressed by the variation in the association and dissociation rates as obtained from noise analysis and reflected by current kinetics. While saturation, competition, or block are sufficiently explained (Läuger, 1973; Hille, 1975) by

an obligatory interaction of one ion with at least one or several polar channel sites ("single-file" condition), findings like the anomalous mole-fraction relationship imply the possibility for multiple occupation of a channel having more than one site (Hille & Schwarz, 1978; Urban & Hladky, 1979) thereby allowing various interactions between neighboring ions. The simplest model that can accommodate the above findings is a channel with at least two sites (energy wells), separated from each other and the adjacent solutions by energy barriers. Such a "two-sites/three-barriers" (2S3B) model describes the properties of the gramicidin channel (Urban & Hladky, 1979; Finkelstein & Andersen, 1981) and also the K^+ channel in membranes of nerve and muscle (Hille & Schwarz, 1978), and of the starfish egg (Hagiwara et al., 1977).

Ion Transport Parameters: The permselectivity problem. In a previous publication (Zeiske & Van Driessche, 1979) we surveyed the literature dealing with the cation permeability other than for Na^+ of the apical membrane in frog skin. For intermoult phases, in adult *Rana temporaria,* a significant K^+ permeability was reported only recently (Zeiske & Van Driessche, 1979; Nagel & Hirschmann, 1980). Furthermore, some permeability for NH_4^+ and Tl^+, besides that for Na^+, was observed in adult *Rana catesbeiana* (however, not in all animals) while no K^+ permeability was found (Benos, Mandel & Simon, 1980). On the other hand, noise analysis revealed the existence of rather nonselective monovalent cation channels in the apical membranes of larval bullfrog skin (Zeiske, Hillyard & Van Driessche, 1982).

From the experiments reported here we conclude that, besides K^+, also the "K^+-like" cations NH_4^+, Rb^+ and Tl^+ do pass the Ba^{2+}-blockable, spontaneously fluctuating K^+ channel. So far our results seem to conflict with a previous statement (Van Driessche & Zeiske, 1980*a*) that no spontaneous noise could be observed with NH_4^+ and Rb^+. At that time probably not enough comparative experiments had been performed. It becomes clear from this paper that the chances to find appreciable ion-specific currents with NH_4^+ or Rb^+ are considerably worse than for K^+ or Tl^+ (*cf.* Fig. 1). This is even more true for Lorentzian spectra.

The K^+-like ions can also permeate the K^+ channel in the squid axon (Hille, 1975), the node of Ranvier (Hille, 1973) and the skeletal muscle membrane (Coronado, Rosenberg & Miller, 1980; Stanfield, Ashcroft & Plant, 1981). While a qualitative statement about the frog skin's K^+-channel selectivity thus seems unproblematic and shows analogies to other K^+ channels, quantitative statements like "selectivity order" or "permeability ratios" appear ambiguous or even impossible. The reason for this lies in the multi-site single-file behavior: Allowing multiple occupancy creates severe problems in predicting, for a series of ions, permeability or conductance ratios both of which now depend on the activities of the permeant ions in the solutions at both sides of an ionic channel (Sandblom et al. 1977; Hille & Schwarz, 1978; Urban & Hladky, 1979). Generally no details are known about the factors governing ion transport in such a case; e.g. voltage- and occupancy-dependent rate constants for ion "hopping" over the barriers, or effects on the energy-well depth.

In the investigated concentration range the order of current magnitude ($NH_4^+ < Rb^+ < K^+ < Tl^+$) is similar to the one given for K^+-channel selectivities in excitable membranes (Hagiwara et al., 1974, 1977; Hille, 1975; Reuter & Stevens, 1980). Contrary to those studies, we do not know the actual apical membrane voltage nor the exact chemical driving force when different ionic conditions are tested and are to be compared. Thus we cannot evaluate single-channel conductances from noise analysis.

The channel occupancy. The apparently simple saturation behavior of the K^+ and Tl^+ currents might well indicate a single occupancy of a multisite K^+ channel by either of these ions. However, deviations from simple saturation at very high ionic activities cannot be revealed by extrapolating an apparent saturation behavior observed in a lower limited concentration range. While the linearity in the graphical analysis of the current-concentration relationship (Fig. 2) seems to be preserved with K^+ and Tl^+ up to Ringer's concentrations, this is not the case with Rb^+ and certainly does not hold for NH_4^+. Particularly the latter ion has the possibility for sterically directed binding in contrast to the other, monoatomic, cations. That might play a role for the observed strong expression of cooperative interaction with the K^+ channel. Another possible reason for the deviation from simple saturation might be an effect of small amounts of H^+ originating from the dissociation of NH_4^+ in poorly buffered regions near the membrane, or in the channel vicinity. However, the analogous finding of, although weakly indicated, cooperativity with Rb^+ does not support the latter hypothesis. Especially the anomalous mole-fraction effect, and the remarkable dependence of the Ba^{2+} block on the permeating ion species (*see be-*

low) cast doubt on the assumption of single occupation with whatever ion present.

Anomalous mole-fraction relations showing a minimum, as those we observed have been reported for K^+/Tl^+ mixtures with membranes in the starfish egg cell (Hagiwara et al., 1977), as well as with the ionophore gramicidin incorporated in lipid bilayers (Neher, 1975; Neher, Sandblom & Eisenman, 1978). This phenomenon has been interpreted as indication of a multi-site single-file channel where multiple occupancy by different permeant ions influences the rate constants for hopping across one or more of the energy barriers. For instance, a strong association of Tl^+ at a more inner site (competitive with K^+) will slow the entrance, but speed up the exit rate for K^+ at the neighboring outer channel mouth. With a large K^+/Tl^+ ratio the total current which will be mostly carried by K^+ will be smaller than with K^+ alone. At high Tl^+/K^+ ratio the strong Tl^+ interaction at the inner site will be reduced by the strong Tl^+ interaction at the outer channel mouth, so the current, now mainly carried by Tl^+, will increase again. Hille and Schwarz (1978), however, pointed out that anomalous mole-fraction relations need not have a minimum. Rather, the constellation of (probably unsymmetrically distributed) barrier heights and well depths can virtually produce a multitude of shapes in the mole-fraction relation. On the other hand, minima only have so far been described for different experimental systems, and always with K^+/Tl^+ mixtures. Certainly, the fact that Tl^+ has a strongly polarizable d-electron shell (in contrast to the other "K^+-like" cations) facilitates intimate interactions with negatively charged, or other polar, regions in the channel.

In this context, the fast negative current transients seen during the substitution of one permeant ion by another (Fig. 1) deserve some comment. During solution exchange we have to assume that a continuous front of the "new" solution approaches the membrane. Within the distance of the unavoidable unstirred layer, replacement of e.g. K^+ by Tl^+ (the latter having a stronger interaction with the channel) results in a transient solution composition with a high K^+/Tl^+ ratio, thus comparable to the mixture situation around the minimum of the mole-fraction relationship, which could result in an analogous SCC inhibition. Further moving of the Tl^+-solution front will then increase the Tl^+/K^+ ratio with simultaneous current increase. The fast transients may therefore be interpreted as a kind of "fast-mole fraction experiment," especially because they are not observed when Ba^{2+} blocks the K^+ channel. However, any other interpretation of the transient cannot be excluded at this point.

Spontaneous Fluctuations of the Multi-Site Channel

First of all, there seems to be no doubt that the K^+-like cations NH_4^+, Rb^+ and Tl^+ pass the fluctuating K^+ channel. The findings from studies of the macroscopic current, like competitive effects among permeant ions, or the blockade of the ionic current by mucosal Ba^{2+}, are excellently mirrored by noise analysis: spontaneous Lorentzian noise with either permeant cation; Lorentzian plateaus varying similarly as currents (with the exception of Rb^+) in pure Ringer's, as well as, although with some reservations, in K^+/Tl^+ mixtures; a Ba^{2+}-dependent induction of a second Lorentzian, blocker-type, noise component in all cases (for a more thorough discussion of the Ba^{2+}/K^+ channel interaction, *see below*). Finally, the toxic long-term effects of high Tl^+ concentrations show up in current and noise data as well.

A special, since unexpected, feature in the results from noise analysis certainly is the obvious interference of the given permeant ion with the channel-gating process. Not only for the spontaneous fluctuations has such an interference been found but it also seems to exist for the blocker-induced conductance fluctuations (*see* next chapter). Very recently mechanisms have been discussed, for K^+ channels in excitable membranes, how permeant K^+-like ions might affect the lifetime of the open or closed channel-state (Stanfield et al., 1981; Swenson & Armstrong, 1981). For frog skin, mucosa positive potentials have been found (Zeiske & Van Driessche, 1981) to markedly influence K^+-channel gating by increasing the mean time closed. In a multi-site channel one would therefore expect an "internally" located gate to respond similarly to an occupation of a more "externally" located, negatively charged, neighboring site in the pore by a permeant cation. Certainly, the chemical nature of the ion and its proper specific interaction with the channel environment will be reflected by a specific shift in the gating rate of the fluctuating channel, as compared to the unoccupied state.

Ba^{2+} and the Multi-Site Channel

With respect to its crystallographic ion radius (Eaton & Brodwick, 1980), Ba^{2+} can be considered to be a K^+-like cation. It is clear that the double charge will interact very strongly with negatively

charged, or otherwise polar, K^+-channel components. In a previous paper (Van Driessche & Zeiske, 1980*b*) we discussed the "competitive" blockade of the K^+ current by Ba^{2+} at length. It was also shown that the blocker-receptor interaction is reflected by the rise of a Ba^{2+}-dependent, second Lorentzian component in the K^+-current noise, a feature which is equally obtained with other permeant ions as reported here. However, due to the complicated current analysis with Tl^+, NH_4^+ and Rb^+, we could not establish a competition of Ba^{2+} with these ions.

For the Ba^{2+} blockade of NH_4^+ and Rb^+ current, a description by a pseudo "two-state" mechanism is given through the linearity of the $2\pi f_c^{(2)}$-$[Ba^{2+}]$ plots, within the investigated $[Ba^{2+}]$ range. This finding is in accordance with our previous studies concerning the Ba^{2+} blockade of the K^+ current (Van Driessche & Zeiske, 1980*b*). The *de facto,* two-state behavior in the frame of a three-state model for spontaneous and blocker-induced relaxation noise shows that the reaction with the blocker is negligibly influenced by the spontaneous fluctuations. In the reaction scheme proposed by us, Ba^{2+} binds to the open channel conformation. The similarity in corner frequency for the spontaneous fluctuations with either K^+ or NH_4^+ may well indicate that, in both conditions, the open state probability is close to unity (*cf.* Van Driessche & Zeiske, 1980*b*). In such a case, together with the precondition for minimal coupling of spontaneous and blocker-induced noise ($f_c^{(2)} < f_c^{(1)}/2$), we must conclude that, in presence of Ba^{2+}, the corner frequency $f_c^{(1)}$ for the spontaneous fluctuations with NH_4^+ is much increased. In contrast, the condition $f_c^{(2)} < f_c^{(1)}/2$ is easily met for Rb^+ where $f_c^{(1)}$ is quite large, and the rate constant α_{02} is, as with K^+, much smaller than with NH_4^+.

The Table shows that the Ba^{2+}-association rate constant is much larger for NH_4^+, but comparable in magnitude for K^+ and Rb^+. The difference in the Ba^{2+}-dissociation rate constant is much less expressed for these three ions but still amounts to a factor of 11 between K^+ and Rb^+, and of 5 between K^+ and NH_4^+. One could easily come to the conclusion that the accessibility of the Ba^{2+} receptor which is thought to mediate the Ba^{2+}-dependent modification of the spontaneous fluctuations, parallels the accessibility of the inhibitory site for Ba^{2+} ions. This would imply two receptors for Ba^{2+}: One (the selectivity filter) would be responsible for the channel block, whereas the other (probably located more externally at the channel mouth) would not result in blockage but influence the spontaneous gating by short-range forces.

Table.

	K^+	Rb^+	NH_4^+	Tl^+
α_{02}	0.28	0.13	7.0	very small?
α_{20}	22.0	250.0	100.0	5
K_{Ba}	80	2000	15	very large?

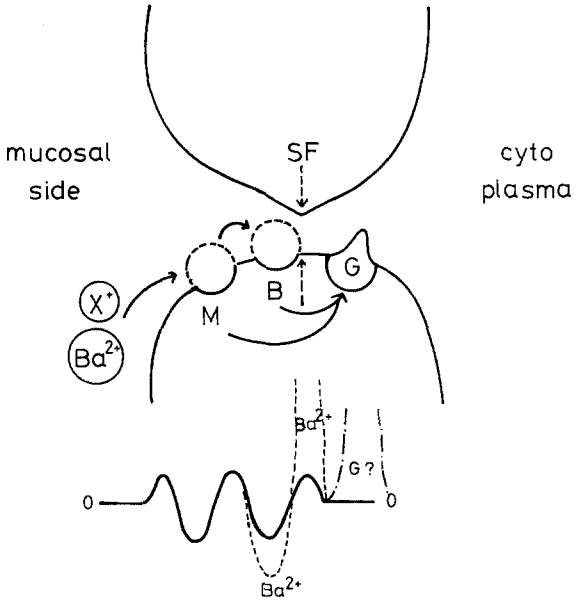

Fig. 9. Hypothetical model of a 2S3B K^+ channel. *M* and *B* denote the sites for interaction with permeant ions (X^+) and Ba^{2+} which "hop" from the solution and along the single-file sites. Appropriate interaction of X^+ and/or Ba^{2+} at *M* (modifier site) and/or *B* (blocker site) influences the kinetics of the channel gate *G*. A selectivity filter *SF* allows the passage of only X^+ (NH_4^+, Rb^+, K^+, Tl^+) but not Ba^{2+} to the cytoplasmic channel side. A tentative potential profile is given in the lower panel. Barrier heights and well depths are, for simplicity, assumed to be uniform. The profile does not reflect any electrical transmembranal potential

Our preliminary findings point toward very slow rates for Ba^{2+} association and dissociation in presence of Tl^+ (Table). In the case of "competitive" interactions of $Ba^{2+}/K^+/Tl^+$ within the channel we would understand the very small Ba^{2+}-receptor association rate (α_{02}) and the bad efficacy as a blocker in the presence of Tl^+: A strong interaction of Tl^+ with the channel (*cf.* Fig. 3) will reduce very much the chance for Ba^{2+} for a similar interaction. On the other hand, once bound Ba^{2+} would leave the channel very slowly if it had to exit from the single-file path by forcing the strongly interacting Tl^+ from the neighboring site back into solution. An analogous mechanism may then explain the variation of α_{02} and α_{20} for Rb^+, NH_4^+ and K^+ as permeant ion species.

To summarize (*cf.* Fig. 9; further details in legend) we suggest that the selectivity filter discrimi-

nating between Ba^{2+} and the permeant ions lies more towards the cytoplasmic channel mouth, and that the influence of the permeant ions on the Ba^{2+}-receptor interaction and on the spontaneous channel gating is exerted from an appropriate cation being at a more external single-file position. In such a case, the rate of access of Ba^{2+} to the selectivity filter would increase, the less the permeant ions interact with the outer site. Then also the exit of Ba^{2+} back to the solution will occur faster. The extended version of the reaction scheme presented earlier for the description of the $Ba^{2+}-K^+$ channel interaction (Van Driessche & Zeiske, 1980*b*) would be:

$$\text{blocked} \underset{\alpha^*_{20}}{\overset{\alpha^*_{02}[Ba^{2+}]}{\rightleftharpoons}} \text{open} \underset{\alpha^*_{10}}{\overset{\alpha^*_{01}}{\rightleftharpoons}} \text{closed}.$$

The asterisks denote the sensivitiy of the spontaneous gating (α_{01}, α_{10}) to any K^+-like cation, and that of the blocker-dependent noise (α_{02}, α_{20}) to permeant K^+-like ions.

Frog Skin K^+ Channel Compared to K^+ Channels in Other Membranes

The K^+ channel in the apical membrane of the frog skin bears many similarities to K^+ channels in other epithelial membranes of apical (Van Driessche & Gögelein, 1978; Zeiske, Van Driessche & Machen, 1980; Wills, Zeiske & Van Driessche, 1982; Zeiske, Hillyard & Van Driessche, 1982) and basolateral origin (Van Driessche et al., 1981). Among epithelial K^+ channels the one in frog skin seems the best known today but considering the comparably small amount of information about it, the degree of similarity with K^+ channels in excitable membranes is already amazing:

(*i*) Only monovalent "K^+-like" ions permeate.

(*ii*) The same blockers are found among inorganic ions such as Cs^+, Ba^{2+} and H^+. However, TEA which has shown blocking action of K^+ movement in several other epithelial K^+ channels (Van Driessche & Gögelein, 1978; Wills, Zeiske & Van Driessche, 1982; Zeiske, Hillyard & Van Driessche, 1982) does not block the K^+ channel in adult but in larval (Zeiske, Hillyard & Van Driessche, 1982) frog skin. For most K^+ channels, the site for interaction with competitive but impermeant blockers is thought to be located more inside the channel (Hille, 1975; Eaton & Brodwick, 1980) as we proposed for our system, too.

(*iii*) The K^+-channel gate which triggers the spontaneous conductance fluctuations can be influenced by electrical fields of various origin. In nerve membranes the gate is thought to be more towards the cytoplasmic channel mouth (Hille, 1973, 1975) which agrees with our view of the frog skin's K^+ channel.

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