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SPONTANEOUS FLUCTUATIONS OF POTASSIUM CHANNELS IN THE APICAL MEMBRANE OF FROG SKIN

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SUMMARY

1. The previously demonstrated K^+-dependent short-circuit current through the skin of the frog species *Rana temporaria* (Zeiske & Van Driessche, 1979), bathed with mucosal K^+- and serosal Na^+-Ringer solution, was investigated with current-fluctuation analysis.

2. The current-noise spectra were recorded in the frequency range from 1 to 800 Hz and showed a Lorentzian component with a mean plateau value $S_0 = (1{\cdot}50 \pm 0{\cdot}05).10^{-20}$ $A^2.s.cm^{-2}$ and a corner frequency of $f_c = (81{\cdot}0 \pm 3{\cdot}4)$ Hz ($n = 14$).

3. S_0 increased with mucosal K^+ concentration, $[K]_o$, while f_c remained almost unchanged. A decrease in S_0 was observed when serosal Na^+ was replaced by K^+.

4. Mucosal Cs^+ (10 mM) depressed, reversibly, the K^+-dependent current noise to the level of the background noise. Moreover, a linear decrease in f_c with increasing Cs^+ concentration was observed.

5. Among the other tested alkali cations, Rb^+ was the only blocker though less potent than Cs^+. Tetraethylammonium, 4-aminopyridine, 2.4.6-triamino-pyrimidine and amiloride had no effect.

6. Alterations in the transcellular transport of Na^+ contained in a mucosal solution with high $[K]_o$ resulted in significant changes in K^+ current noise.

7. The current-fluctuation intensities decreased with increasing contact time to high $[K]_o$; these changes were concomitant with the previously reported time dependence of the short-circuit current (Zeiske & Van Driessche, 1979).

8. The K^+-dependent fluctuations are thought to originate from K^+-selective pathways in the apical cell membranes. The description of the K^+-current noise by a single Lorentzian suggests that the 'K^+ channels' switch randomly between an open and closed state.

9. Assuming a two state model for the channel-kinetics, the single channel current i and the channel density M were calculated as $i = (0{\cdot}37 \pm 0{\cdot}05)$pA and $M = (0{\cdot}53 \pm 0{\cdot}08)$ μ^{-2} ($n = 13$).

INTRODUCTION

The outer membrane of the frog skin is thought to be highly permselective for Na^+ ions (Koefoed-Johnsen & Ussing, 1958). Recently a K^+-selective pathway across the outer skin border of *Rana temporaria* has also been described. The kinetic characteristics were similar to that known from apical Na^+ channels (Zeiske & Van Driessche, 1978*a*; Zeiske & Van Driessche, 1979). A linear relationship between the logarithm of

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the mucosal K^+ concentration and the transepithelial potential (outside negative) was demonstrated, and a saturable K^+-dependent short-circuit current was found which was depressed by mucosal Cs^+ ions. Micro-electrode investigation (Hirschmann & Nagel, 1978) suggested that the K^+-specific structures were located in the outer membranes of the first living cell layer.

We studied the nature of these K^+ pathways with fluctuation analysis of the K^+-dependent short-circuit current. It may be concluded from our experiments that the K^+-selective structures are membrane-bound ionic channels which open and close randomly. Some of the results were presented at the winter meeting of the Belgian Physiological Society, Brussels (Van Driessche & Zeiske, 1978) and the spring meeting of the Deutsche Physiologische Gesellschaft, Göttingen (Zeiske & Van Driessche, 1978*b*).

METHODS

Abdominal skins of the frog species *Rana temporaria* were mounted in an Ussing-type lucite chamber. To avoid edge damage the skin was sealed by soft silicon-rubber rings (Silgel 604: 97 % part A; Wacker-Chemie, München, F.R.G.). The skin area exposed to the bathing solutions was 0·126 cm^2. Rapid solution changes were made by means of a syringe. Changes of spontaneous potentials or of the short-circuit current could be observed immediately, especially the quick inhibitory effect of Cs^+ (cf. Zeiske & Van Driessche, 1979, Fig. 2). However, the time needed to adjust the experimental set-up for the noise measurement was never less than 30 sec. For this reason the effect of solution changes on K^+ noise could only be observed after this delay. Because of the long-time effect described in a previous paper (Zeiske & Van Driessche, 1979), a correlation or comparison of transepithelial electrical parameters with noise data is not meaningful and therefore not discussed.

The electronic set-up for low-noise amplification of the short-circuit current and the method of recording and processing the data are described in detail elsewhere (Van Driessche & Lindemann, 1978). Briefly, the amplified current fluctuations were filtered by a low- and a high-pass filter with cut-off frequencies of 850 and 0·1 Hz, respectively. The filtered analog signal was digitized (sample rate 0·5 msec), stored in a buffer-memory and transferred to a digital magnetic tape. Sixty data blocks of the current-noise signal (each of 1·024 sec length) were analysed by a fast Fourier transform routine (DECUS number 179) on a computer (Digital Equipment PDP 11/34). The final power density spectra were normalized for 1 cm^2 skin area and represent the average of the sixty data blocks. For the observation of the long-time effect of the high mucosal K^+ concentration only thirty data blocks were recorded with a sample rate of 0·1 msec. The total time needed for recording and writing the data on magnetic tape was 30 sec. Therefore each dot in Fig. 6*B* represents an average of 30 sec recording time, e.g. '5 min' on the abscissa indicates a recording interval between 4 min 45 sec and 5 min 15 sec.

The spectra were displayed on the monitor (VR 17) of a graphic display processor (VT 11, Digital Equipment), photographed and redrawn. Plateau values and corner frequencies of the Lorentzian component in the spectra were obtained in the following way: a computer-generated Lorentzian curve was displayed on the screen and moved by computer commands until it was observed that the best fit to the experimental data was obtained. For some skins this procedure may be more critical at spectral densities below 5×10^{-21} $A^2.sec.cm^{-2}$. Some preparations may show such a large background noise that the Lorentzian component reduces to a 'shoulder' in the whole spectrum. Subtraction of the background noise is not possible because the background can also be influenced by the experimental conditions (see, for example, the effect of Cs^+). All reported values of Lorentzian plateaus and corner frequencies were therefore derived from experiments where the determination of the Lorentzian component was sufficiently accurate. This is demonstrated by the fitted spectra in Figs. 2, 4, 5 and 6. Mean values are given $\pm$ S.E. of mean.

The serosal solution was mostly air-bubbled NaCl-Ringer solution containing 2·5 mM-$KHCO_3$, 1 mM $CaCl_2$ and 115 mM-NaCl, pH 8·4. During the recording of the current-noise, the bubbling was stopped. The mucosal solutions consisted of Cl-Ringer solution (pH 7·4) where the con-

centration sum of monovalent cations was held constant at 117·5 mM, unless otherwise noted (details in figure legends). Amiloride was a gift of Merck, Sharp and Dhome, Ltd.

RESULTS

(A) *Lorentzian component in the power-spectrum of the K+-current fluctuations*

Fig. 1*A* demonstrates the fluctuation pattern of the short-circuit current obtained with different mucosal solutions (serosa: NaCl-Ringer solution). With 115 mM-mucosal Na^+ the short-circuit current is 38·9 $\mu A/cm^2$, and its fluctuations around the mean value show an appreciable content of lower frequencies. With 117·5 mM mucosal K^+, however, the current is generally much lower than with Na^+ ions (here 10·4 $\mu A/cm^2$), but its noise shows many high-frequency components. Since it has been shown that mucosal Cs^+ ions rapidly and reversibly depress the K^+-dependent short-circuit current (Zeiske & Van Driessche, 1979), their effect on the current noise is of interest. The addition of 10 mM-CsCl to the mucosal K^+-Ringer solution results in both a quick reduction of the current to 5·3 $\mu A/cm^2$ within 5 sec and a clear decrease in the current fluctuations (Fig. 1*A*).

In Fig. 1*B* the corresponding frequency distribution of the short-circuit current fluctuations shown in Fig. 1*A* is displayed in a so-called 'power spectrum'. Here the spectral density of the current noise is represented in a double logarithmic plot as a function of the frequency. The spectrum shows high intensities at lower frequencies

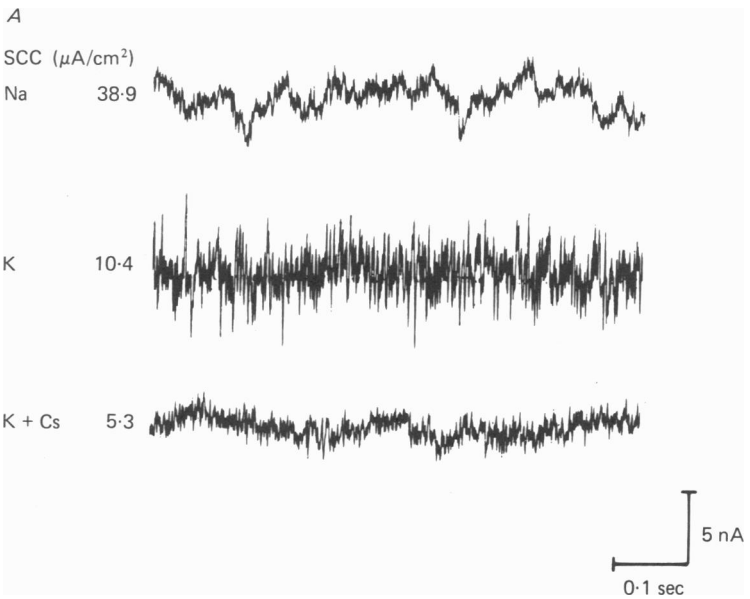

Fig. 1. *A*, fluctuation pattern of the short-circuit current (SCC) around its mean value (figures left) for mucosal NaCl-Ringer (Na), KCl-Ringer (K), and 10 mM-CsCl containing KCl-Ringer (K + Cs). Serosal: NaCl-Ringer. *B*, power spectra of the short-circuit current fluctuations from *A*, with △ for Na, ○ for K and □ for K + Cs. A Lorentzian was fitted to the middle part of the K^+ spectrum with the Lorentzian-plateau value S_0 and the corner frequency f_c.

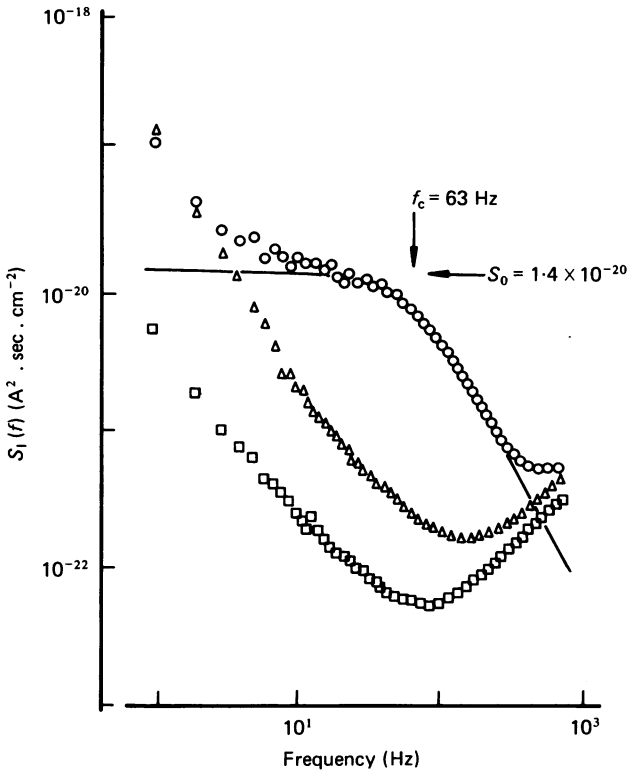

Fig. 1. *B*

(< 10 Hz) in the case of mucosal Na^+, and the curve is almost linear between 1 and 30 Hz, with a slope of about -2. For mucosal K^+, the spectral density at frequencies above 10 Hz is appreciably larger and decreases with a slope of -2 in the range from 90 to 300 Hz. At frequencies below 70 Hz, however, a flattening of the spectrum can be seen which, in some preparations, extends to a short plateau. At higher frequencies the observable increase of the spectral density is due to the amplifier-generated noise which is inversely proportional to the impedance of the preparation (Fishman, Poussart & Moore, 1975). The middle part of the K^+ spectrum may be fitted with a so-called Lorentzian function of the general form (Verveen & DeFelice, 1974):

$$S = S_0/(1 + (f/f_c)^2). \quad (1)$$

Here, S represents the spectral density, f the frequency, S_0 the plateau value or the spectral density at the low-frequency limit, and f_c the corner frequency, where S drops to its half-maximal value $S_0/2$. The underlying mechanism of the Lorentzian-type current fluctuations is generally accepted to be an 'open-close' mechanism of specific ion-transporting structures (Verveen & DeFelice, 1974). The observed macroscopic short-circuit current is thought to be the sum of the elementary currents through these structures which open and close randomly. Consequently the current must fluctuate around its mean value.

In the presence of 10 mM-CsCl the spectral density of the Lorentzian component in the fluctuations of the K^+ short-circuit current reduced 10–100-fold, and the typical curvature disappears. The fluctuations observed at frequencies below 10 Hz in the presence of K^+ ions could also be depressed by Cs^+ ions. The underlying mechanism of this noise source is unknown at present.

A Lorentzian-type frequency distribution was never obtained with mucosal Ringer-solutions containing only Rb^+, Cs^+ or NH_4^+ as the main monovalent cation. Also with mucosal Na^+ or Li^+ in the presence of 50 μM-amiloride, which eliminates the apical uptake of these ions through the Na^+ channels (Ehrlich & Crabbé, 1968; Leblanc, 1972), no Lorentzian could be seen. Except with pure K^+- or Na^+-Ringer solution, the spectral density with mucosal cations was similar to that shown for the Cs^+-containing K^+-Ringer. Furthermore, the addition of 50 μM-amiloride to the mucosal K^+-Ringer solution did not influence the power spectrum of the K^+-current noise. Consequently, the appearance of the Lorentzian component in the current noise depends on the presence of K^+. We found a mean plateau value of the Lorentzian component of $S_0 = (1{\cdot}50 \pm 0{\cdot}05).\ 10^{-20}$ A^2.sec.cm^{-2}, and a mean corner frequency of $f_c = (81{\cdot}0 \pm 3{\cdot}4)$ Hz ($n = 14$).

All the current changes related to $[K]_o$ occurred within seconds. Equally rapid was the inhibitory Cs^+ effect. In particular the full reversibility of the Cs^+ action on the K^+ current and the K^+ noise suggests a localization of the fluctuating K^+-permeable structures at the outer skin border.

In a few cases (about 5 % of all tested skins) experiments with skins of the frog species *Rana esculenta* showed a small K^+-dependent Lorentzian component in the power spectrum though the short-circuit current was practically zero with mucosal K^+-Ringer solution. A K^+-dependent Lorentzian was never observed with toad urinary bladder.

Like the transepithelial potential difference (p.d.) and the short-circuit current (Zeiske & Van Driessche, 1979), the current fluctuations are also strongly dependent on the mucosal K^+ concentration, $[K]_o$. It can be seen from Fig. 2 that the plateau values of the Lorentzian curves increase steeply with increasing $[K]_o$. S_0 could not be determined for $[K]_o < 40$ mM because the fluctuation intensity did not exceed the background noise sufficiently to enable a reasonable fitting procedure of the spectrum by a Lorentzian function. In contrast to the over-all increase in S_0 with $[K]_o$, the corner frequency remained almost unchanged.

In summary, an inward directed short-circuit current together with the existence of a Lorentzian current-noise component points towards K^+-specific pathways, which open and close randomly, in the outer skin border.

(B) *Influence of monovalent inorganic and organic cations on the K^+-current noise*

Like Cs^+ ions, Rb^+ ions were also able to depress the intensity of the K^+-current fluctuations. From Fig. 3 it can be seen that the inhibitory effect of 10 mM-RbCl in the mucosal KCl-Ringer was less marked than with CsCl. In this case, the reduction of the K^+-dependent short-circuit current by Rb^+ was also about half that obtained with Cs^+ ions. Mucosal Li^+ and Na^+ (10 mM in presence of 50 μM-amiloride) did not influence the K^+-dependent Lorentzian component. Similarly the pseudo-alkali ion NH_4^+ (10 mM mucosal) did not change the spectrum of the K^+ noise.

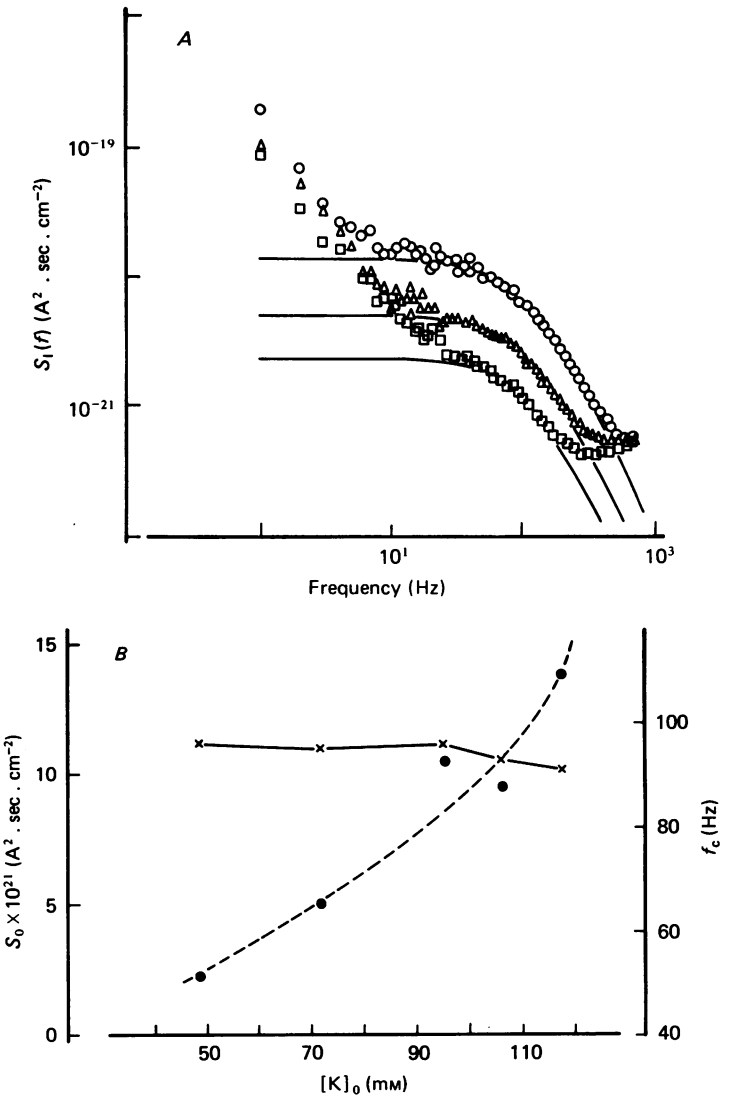

Fig. 2. *A*, power spectra and Lorentzian fits for different mucosal $[K]_o$, with □ for 49, △ for 72, ○ for 117·5 mM-$[K]_o$. For clarity, the spectra with $[K]_o = 96$ and 107 mM are omitted. Solutions: mucosal, K^+- and choline-chloride Ringer the sum of K^+ and choline concentration being always 117·5 mM; serosal, NaCl-Ringer. *B*, plateau values S_0 (●) and corner frequencies f_c (×), of the K^+-dependent Lorentzian from (*A*) as function of the mucosal K^+ concentration, $[K]_o$.

Besides amiloride other cations which are known to block ion transfer were also tested. At pH 6·0, 10 mM-2.4.6-triaminopyrimidine (TAP) which has been found to inhibit paracellular Na^+ movement in gall-bladder (Moreno, 1974) and transcellular Na^+ movement in frog skin (Zeiske, 1976), was without effect on the K^+ current and its fluctuations. Tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP), which are both K^+ blockers in other tissues (Ulbricht, 1977), had also no effect.

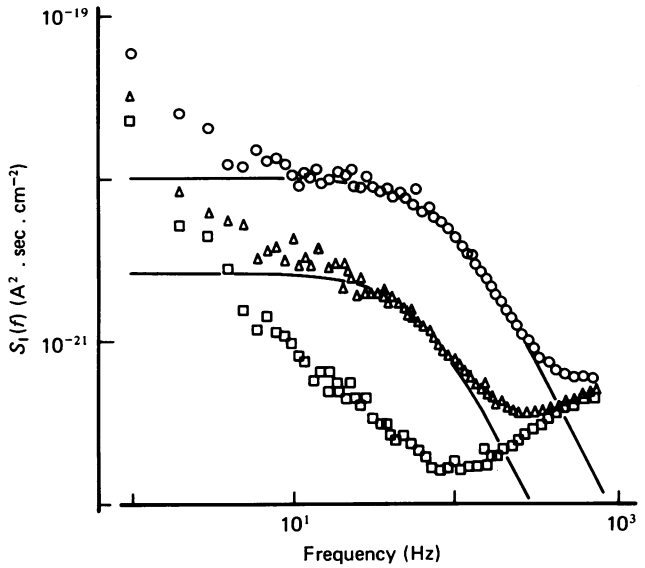

Fig. 3. Influence of 10 mM-mucosal RbCl (△) and CsCl (□) in KCl-Ringer on the $[K]_o$-dependent power spectrum (○). The two upper spectra were fitted by Lorentzians. The lower Lorentzian represents a tentative fit. Solutions: mucosal, KCl-Ringer ± RbCl or CsCl; serosal, NaCl Ringer.

Fig. 4 shows the spectra, plateau values and corner frequencies of the K^+ Lorentzians as function of the mucosal Cs^+ concentration, $[Cs]_o$, for a representative skin. Already at $[Cs]_o > 5$ mM, the Lorentzian component was almost masked by the background noise, and it was not possible to fit a curve. Not only the plateau values S_0, but also the corner frequencies decrease under the influence of Cs^+. The Lorentzian plateaus decrease from $15{\cdot}6 \times 10^{-21}$ $A^2.sec.cm^{-2}$ for $[Cs]_o = 0$ mM to $4{\cdot}2 \times 10^{-21}$ $A^2.sec.cm^{-2}$ with 5 mM-$[Cs]_o$. The decrease in f_c from 85 to 67 Hz seems to be a linear function of $[Cs]_o$.

(C) *Alterations of driving forces and apical membrane resistance: their influence on the K^+-current fluctuations*

In a previous paper (Zeiske & Van Driessche, 1979) we described the decrease in the K^+ short-circuit current with increasing serosal K^+ concentration, $[K]_i$. A similar decrease in the current noise intensity would be expected. A gradual substitution of serosal Na^+ for K^+ led to a reduction of the K^+ Lorentzian. Already with about 50 mM serosal K^+ concentration the Lorentzian disappeared into the background noise (Fig. 5).

Thus it is possible that a K^+-dependent transepithelial chemical gradient drives the inward directed K^+ current either along a transcellular route or via the tight junctions. Reversing the K^+ concentration gradient by using K^+-Ringer solution as the serosal and choline- or amiloride-containing Na^+-Ringer solution as the mucosal medium never yielded any significant short-circuit current or Lorentzian component in the spectrum. This however, is contradictory to the idea of a K^+ current driven by a concentration gradient.

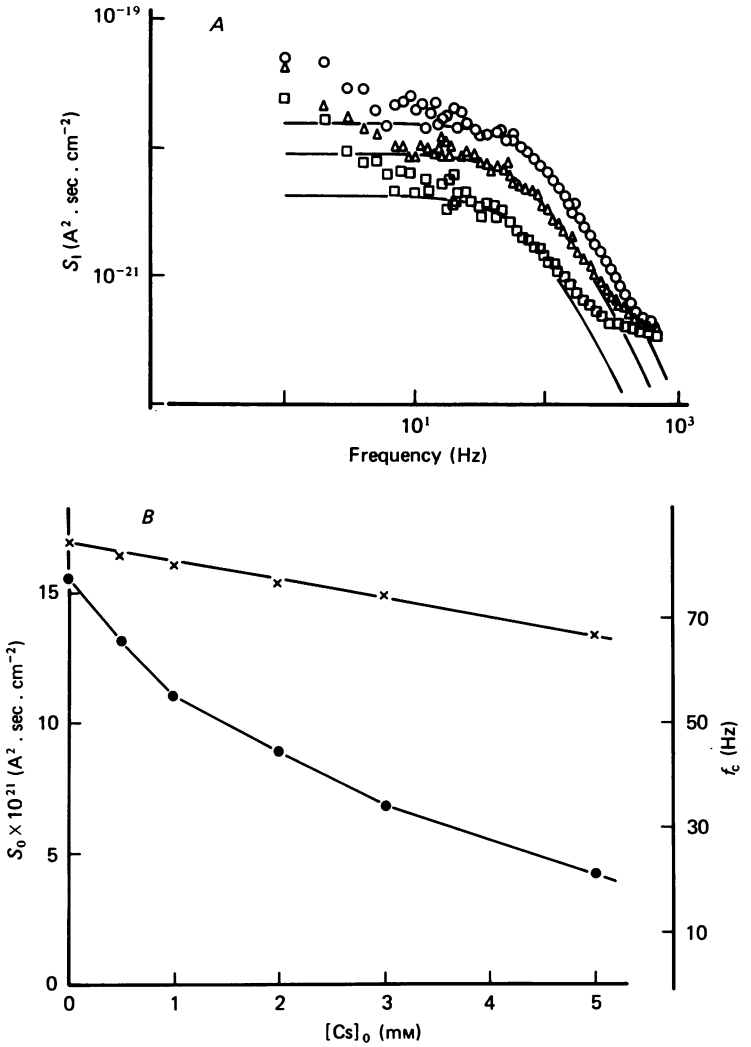

Fig. 4. *A*, power spectra and Lorentzian fits at different mucosal Cs^+ concentrations, $[Cs]_o$. For clarity only three out of six spectra in the range $0 < [Cs]_o < 5$ mM are shown, with ○ for 0, △ for 2 and □ for 5 mM-$[Cs]_o$. Solutions: mucosal, KCl-Ringer ± CsCl; serosal, NaCl-Ringer. *B*, plateau values S_0 (●) from *A* and corner frequencies f_c (×) of the $[K]_o$-dependent Lorentzians as function of the mucosal CsCl concentration.

Though amiloride shows no influence on the K^+ noise in pure mucosal K^+-Ringer solution, an effect on the K^+ Lorentzian is observed if Na^+ ions are present in the K^+-containing solution. In ten skins a reversible increase in the Lorentzian plateau values between 20 and 140 % was observed when 50 μM-amiloride were added to the outer solution containing 94·5 mM-K^+ and 23 mM-Na^+. The average increase in S_0 was from $(4{\cdot}43 \pm 0{\cdot}57) \times 10^{-21}$ $A^2.sec.cm^{-2}$ without amiloride to $(7{\cdot}85 \pm 1{\cdot}53) \times 10^{-21}$ $A^2.sec.cm^{-2}$ with amiloride ($n = 10$). The result is statistically significant according

to the Student's t test with $P < 0{\cdot}05$. A similar and equally reversible behaviour was seen when Na^+ was replaced by choline.

Amiloride affects the transcellular Na^+ transport (Ehrlich & Crabbé, 1968) and therefore all Na^+-dependent electrochemical parameters. The same effect can be

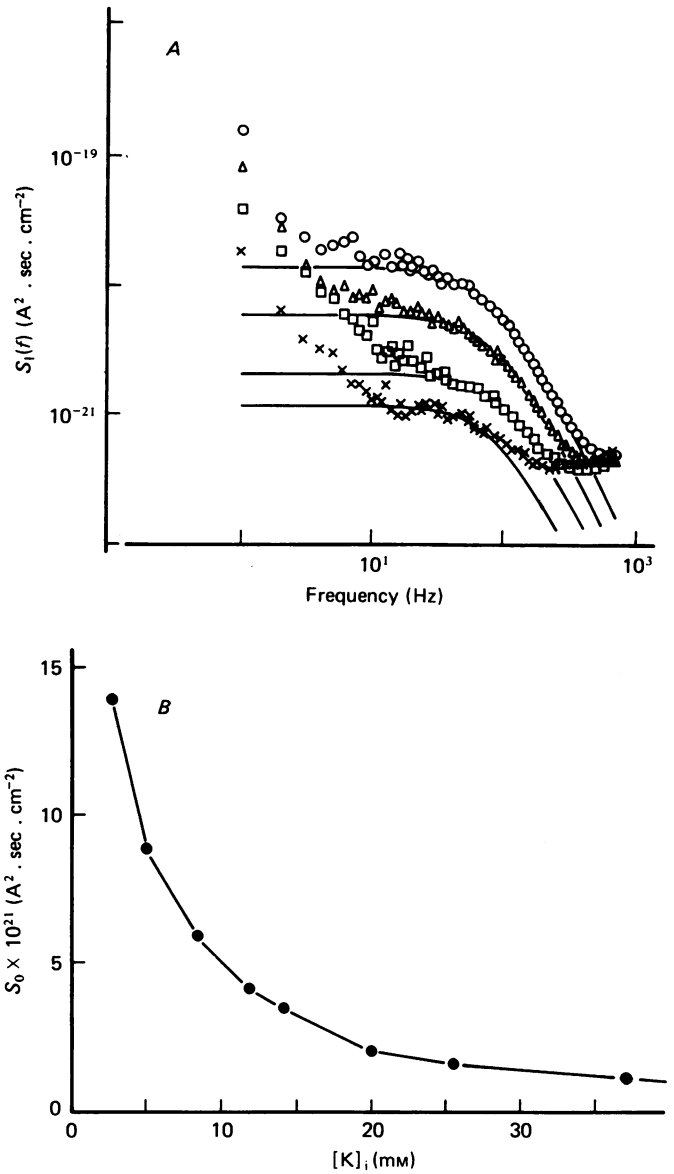

Fig. 5. *A*, power spectra and Lorentzian fits at different serosal K^+ concentration, $[K]_i$. For clarity only four of eight spectra in the range $2{\cdot}5 < [K]_i < 37$ mM are shown, with ○ for 2·5, △ for 8·3, □ for 19·8 and × for 37 mM-$[K]_i$. Solutions: mucosal, KCl-Ringer; serosal, chloride Ringer with K^+ and Na^+ (concentration sum always 117·5 mM). *B*, dependence of the plateau values S_0 of the $[K]_o$-dependent Lorentzians on the serosal K^+ concentration.

expected for the substitution of Na^+ by the impermeable choline. The shifts of the Lorentzian component in the power spectrum should therefore be caused by changes in the apical membrane resistance and/or electrochemical gradients across the apical cell membrane.

(D) *The 'long-time effect' of high* $[K]_o$

Zeiske & Van Driessche (1979) reported that, after a rapid change from mucosal choline- to K^+-Ringer the initially constant short-circuit current began to rise again after about 30 sec. A new steady state value (sometimes 30 % higher) was reached within a few minutes. Concomitant changes in the fluctuation intensity of the short-circuit current are therefore expected. Fig. 6 demonstrates that during the secondary slow current rise the S_0 values of the K^+ Lorentzians decrease with increasing contact time to high $[K]_o$. If, in place of choline, Na^+ is replaced by K^+, the initial S_0 values are much higher. However, the final plateaus (after 15–20 min) seem to reach the same value. That active Na^+ transport may be responsible for the much higher K^+-current fluctuation after a Na^+-K^+ substitution, is suggested by the results of the following experiments: when Na^+ transport was suppressed by 50 μM-amiloride before the Na^+-K^+ substitution, the time course of S_0 was equal to that obtained after the choline-K^+ change. Furthermore, if the transcellular Na^+ movement was stopped by the addition of 10^{-4} M-ouabain to the serosal solution, the time course of S_0 after a Na^+-K^+ change (no amiloride present) was also equal to that after the choline-K^+ substitution. Ouabain did not, however, affect the K^+-dependent current noise after a Na^+-free pre-equilibration.

DISCUSSION

(A) *The interpretation of the Lorentzian component in the noise of* K^+ *short-circuit current*

The general assumption that the apical membrane of the frog skin is practically impermeable for K^+ ions (Koefoed-Johnsen & Ussing, 1958) has recently been challenged for the species *Rana temporaria*: detailed studies of the dependence of transepithelial electrical parameters on mucosal K^+ ions have been published by Zeiske & Van Driessche (1979), and intracellular recordings were done by Hirschmann & Nagel (1978). Briefly, Zeiske & Van Driessche (1979) found a K^+-dependent saturating and inward directed short-circuit current, as well as a transepithelial potential difference (mucosa negative). An inhibitory effect of mucosal Rb^+, Cs^+ and H^+ ions on the transepithelial K^+ movement, and the phenomenon of an increased K^+ transport with increasing contact time to mucosal solutions with high K^+ content were described. This was concomitant with a decreased inhibitory effect of Cs^+. A reduction of the transepithelial K^+ concentration gradient likewise reduced the K^+ movement. Hirschmann & Nagel (1978) investigated the K^+ transport with microelectrodes and localized the K^+-selective pathways in the apical cell membranes. In addition they reported a blocking action of mucosal Ba^{2+} on the apical K^+ transfer. Neither group observed these phenomena with the epidermis of *Rana esculenta* or the epithelium of the toad urinary bladder.

Current-fluctuation analysis has become popular during the last years for the study

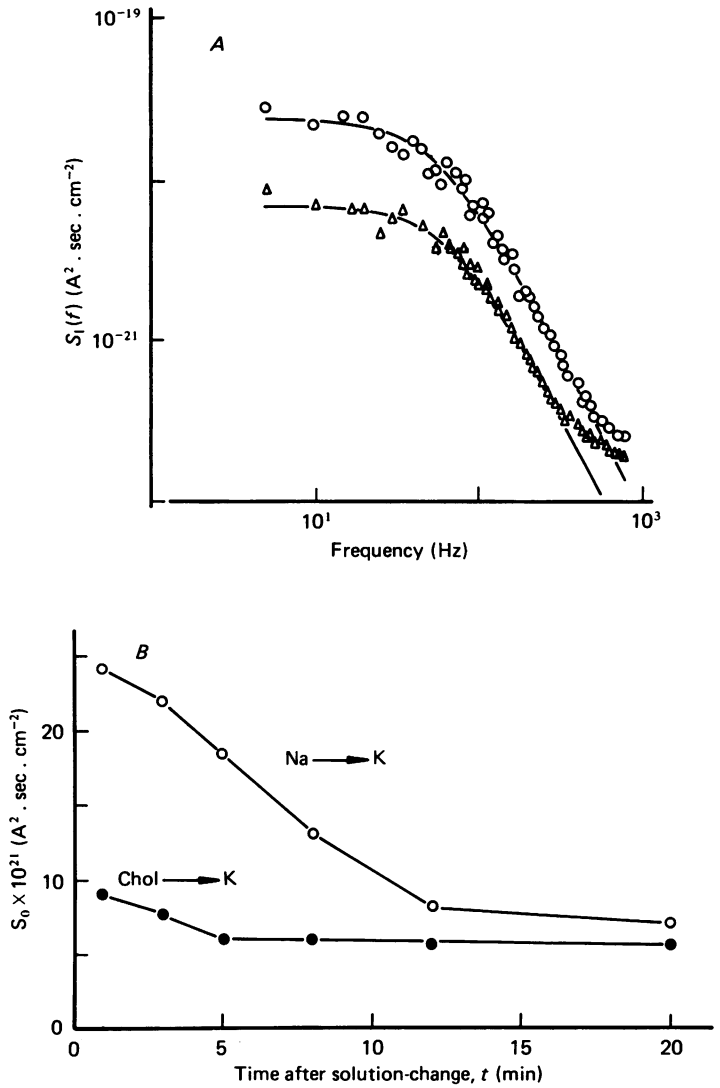

Fig. 6. *A*, power spectra and Lorentzian fits 1 min (○) and 10 min (△) after a change from mucosal Na^+- to K^+-Ringer. For clarity only two out of six spectra recorded during 20 min are shown. *B*, time course of the plateau values S_0 of the $[K]_o$-dependent Lorentzians after a change from mucosal choline-(chol →K), or as in *A*, from mucosal Na^+- to K^+-Ringer (Na →K) in the same skin. Serosal solution, NaCl-Ringer.

of microscopic characteristics of membrane-bound ion transporting structures. Amiloride-induced fluctuations of the Na^+-dependent short-circuit current in frog skin (Lindemann & Van Driessche, 1977) were assumed to be caused by a random switching of the Na^+ pore from an open to a closed state. For a number of ion transport processes across membranes of the muscle synapse, the node of Ranvier and the nerve axon (cf. review by L. J. DeFelice, 1977), as well as across epithelial membranes in amphibian skin (Lindemann & Van Driessche, 1977) and gall-bladder (Van

Driessche & Gögelein, 1978) the 'two-state model' allowed a satisfactory interpretation of the experimental results. This model (Verveen & DeFelice, 1974) assumes a chemical equilibrium between the active and non-active state of the ion transporting structure with the reaction rates α and β. For ionic channels the model describes fluctuations between an open and closed state.

$$\text{open} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \text{closed}. \tag{2}$$

A mathematical analysis of the two-state model (Verveen & DeFelice, 1974) shows that the current noise can be described with a Lorentzian (see eqn. (1))

The Fourier-transformed current fluctuations of the K^+-dependent short-circuit current showed a Lorentzian component (cf. Fig. 1 *B*). In the investigated frequency range such a Lorentzian could never be observed with ions other than K^+ in the mucosal solution. Following the current ideas about the origin of Lorentzian-type power spectra we may assume that the current fluctuations are generated by a channel with 'two-state' kinetics. In the two-state model the chemical rate of the channel kinetics is

$$2\pi f_c = \alpha + \beta. \tag{3}$$

We found no indication for any dependence of f_c (and therefore of the reaction rate) on the K^+ concentration in the mucosal Ringer-solution. The disappearance of the current noise, after abolishing the chemical K^+ gradient over the epithelium (cf. Fig. 5), makes it unlikely that the K^+ noise is caused by an active transport mechanism.

(B) *Where are the fluctuating structures localized?*

Zeiske & Van Driessche (1979) found that the electrical parameters depending on mucosal K^+ changed within seconds after the addition of Cs^+. As already pointed out in Methods, the earliest possible noise-recording was about 30 sec after a solution change. At this time the effect of Cs^+ on the K^+ noise was already fully established. It was reversible within the same time after omitting Cs^+. These observations strongly suggest a localization of the fluctuating structures at the outer skin border.

Furthermore, the amiloride experiments (Results, section *C*) show that the fluctuating channels are not identical with parts of the tight junctions as was presumed previously (Zeiske & Van Driessche, 1978*b*): the apical membrane resistance increases by blocking Na^+ transport with amiloride. Concomitantly the intracellular potential in the short-circuited state becomes more negative (Helman & Fisher, 1977). The increase in the intracellular negativity would only then result in the observed increase in the K^+ current noise if K^+ transport occurs through fluctuating structures in the apical cell membrane. This conclusion would agree with the findings of Hirschmann & Nagel (1978) who recorded intracellular potentials in the short-circuited state which were strongly dependent on the mucosal K^+ concentration.

The fact that it was impossible to obtain a K^+ Lorentzian or even a significant outward directed short-circuit current with a reversed K^+ concentration gradient could be due to a rectification of the K^+ channel permeability. This may have

geometrical reasons, or it may be caused by asymmetric potential barriers at the ends of the K^+ channel. Rectification could also be 'induced' by changes in the intracellular potential after depolarizing the serosal membranes with high $[K]_i$. This would be similar to the potential dependence of the K^+ permeability in excitable tissues. The current–voltage relationships of the K^+-permeable skins (cf. Zeiske & Van Driessche, 1979) show a breakpoint in their slope at zero transepithelial voltage. However, these experiments were done with low serosal, but high mucosal, K^+ concentrations and may not be comparable to the situation of a reversed K^+ concentration gradient. Secondly a rectification of the K^+ channels in the inner membrane cannot be excluded. Finally an increase in the cellular K^+ concentration by high $[K]_i$ could decrease the apical K^+ permeability. Such an effect was described for cellular Na^+ and the apical Na^+ permeability (Shum & Fanelli, 1978).

(C) *The specificity of the apical K^+ channel*

As was already pointed out, mucosal cations other than K^+ produced neither a significant transepithelial current nor a Lorentzian component in the spectrum of the current fluctuations. Also, most of the tested monovalent ions had (up to a concentration of 10 mM in the mucosal solution) no influence on the K^+-dependent current noise. Only Rb^+ which is known as a K^+ substitute in other tissues (Müller, 1965) showed a moderate, and Cs^+ known as a K^+ blocker (Isenberg, 1976), showed a marked inhibitory effect on the fluctuations (cf. Fig. 3). Na^+ ions, Li^+ ions, and the (pseudo-alkali) ammonium ion, did not change the K^+-noise spectra.

Katz (1978) reported an influence of amiloride on K^+-conducting structures during the toad skin moulting cycle and concluded that Na^+ and K^+ pores have similar characteristics during the moult. We never observed a sensitivity of the K^+ fluctuations towards amiloride in Na^+-free mucosal K^+-Ringer. This is consistent with findings from micro-electrode experiments in K^+-permeable skins of *Rana temporaria* (Hirschmann & Nagel, 1978). Furthermore the K^+-permeable structure in frog skin is not sensitive to specific organic blockers of the K^+ channel in excitable tissues, like 4-aminopyridine and tetraethylammonium (Ulbricht, 1977). Thus it must be concluded that the K^+-channel characteristics for the outer membrane of frog skin differ considerably from those in other tissues.

Zeiske & Van Driessche (1979) described in detail the inhibitory effect of mucosal Cs^+ on the kinetics of the transepithelial K^+ movement. It could be shown that, after a short contact time with mucosal K^+, Cs^+ acted as a 'competitive inhibitor'. However, with a longer contact time to mucosal K^+, the inhibitory Cs^+ action decreased more and more while K^+-dependent short-circuit current and conductance increased. This 'long-time-effect' was tentatively interpreted as a 'selectivity-loss' of the K^+-transporting structures. In this paper, we report that after a change from mucosal choline to K^+-Ringer a decrease in the Lorentzian plateaus is observed (cf. Fig. 6*B*, lower curve) which occurs with about the same time course as the concomitant increase in short-circuit current and conductance (cf. Zeiske & Van Driessche, 1979). Since, in this experiment, the skin was pre-equilibrated with Na^+-free Ringer for a long period, Na^+ transport cannot be responsible for this 'long-time effect' of high $[K]_0$. If the K^+-channel permeability increased with longer contact time to mucosal K^+, the increased short-circuit current and the concomitant decrease in

the inhibitory action of Cs^+ would be due to a reduction in Cs^+-blockable spontaneously fluctuating K^+ channels.

A pre-equilibration in Na^+-containing solution, and a subsequent Na^+-K^+ substitution give much higher initial plateau values than without pre-equilibration (Fig. 6*B*, upper curve). However, in each case there was a decrease to almost the same level. In this case a pre-existing Na^+ transport could have had an additional influence on the K^+-dependent current noise.

If the basolateral Na^+ pump were electrogenic as was recently claimed (Nagel, 1978), the difference between the two curves in Fig. 6*B* could be understood. After a Na^+-K^+ substitution, the Na^+ transport compartment would slowly be emptied by the action of the Na^+ pump. At the same time the contribution of the electrogenic pump to the negative intracellular potential in the short-circuited state (V_{SC}) would vanish. The absolute value of V_{SC} should then decrease, as should the driving force for the apical K^+ transfer. Thus the current fluctuations should be reduced.

(D) *A tentative evaluation of single-channel parameters*

Accepting the two-state model for the K^+ channel the experimental values S_0 and f_c are related to microscopic parameters as follows (Lindemann & Van Driessche, 1977):

$$2\pi f_c = \alpha + \beta = 1/\tau, \tag{4}$$

$$S_0 = 4\, M i^2 P_0 P_1 \tau. \tag{5}$$

Here the rate of the channel kinetics, $2\pi f_c$, is equal to the sum of the single rates of the opening (α) and the closing (β) reaction. The inverse of the reaction rate $2\pi f_c$ is called the relaxation time τ of the underlying process. With a mean value of $f_c = 81$ Hz, τ can be estimated to 1·96 msec, a value which is close to that reported for K^+ channels in the squid axon membrane (Fishman, Moore & Poussart, 1975). The plateau value of a Lorentzian calculated from the two-state model is a product of the relaxation time and four unknown factors: the probabilities to find a channel open (P_0) or closed (P_1), the number of spontaneously fluctuating channels (M) and the single-channel current (i) in the open state. The mean K^+-dependent short-circuit current I_K, is then given by

$$I_K = M.i.P_0. \tag{6}$$

Since at present we do not know the probabilities P_0 and P_1, a tentative assumption of equal 'on-and-off' probabilities is made. Inserting eqn. (6) into eqn. (5) and rearranging gives

$$i = \frac{S_0}{2.I_K.\tau}. \tag{7}$$

With the experimental values of S_0, f_c and I_K, the single channel current i may be computed. Using $P_0 = 0{\cdot}5$ and the computed value of i, eqn. (6) yields M, the number of fluctuating K^+ channels. For thirteen skins, i and M were calculated separately. Their mean values are $i = (0{\cdot}37 \pm 0{\cdot}05)$ pA and $M = (0{\cdot}53 \pm 0{\cdot}08)\ \mu^{-2}$. For this series of skins the mean values of I_K, S_0 and f_c were: $I_K = (8{\cdot}9 \pm 1{\cdot}3)\ \mu A.cm^{-2}$, $S_0 = (12{\cdot}1 \pm 1{\cdot}3) \times 10^{-21}\ A^2.sec.cm^{-2}$ and $f_c = (79{\cdot}5 \pm 3{\cdot}3)$ Hz ($n = 13$).

As we already pointed out, a variation of the actual unknown values of P_0 and P_1

(here assumed to be 0·5) can yield appreciable differences for the values of i and M. In addition to that uncertainty, the 'long-time' effect of high $[K]_o$ could lead to an error in the evaluation of i and M. Since the experiments for the determination of i and M were preformed in the late steady-state after a change to high $[K]_o$, the respective I_K values represent a considerable source of error and may be up to 30 % too high (Zeiske & Van Driessche, 1979). If, as suggested above (cf. also Fig. 6), the time-dependent S_0-decrease is due to a decrease in the number of detectable fluctuating K^+ channels, the calculated figures would be an underestimation of i, and an overestimation of M. A correction of I_K for unspecific shunt-currents (mucosal CsCl- or choline chloride-Ringer) which are usually negligible does not lead to significantly different values for i and M.

The analysis of amiloride-induced fluctuations of Na^+ channels in the apical membrane of frog skin showed that a reversible blocker may cause (*a*) a shift in f_c of the 'spontaneous' Lorentzian, dependent on the blocker-concentration and (*b*) the appearance of an additional blocker-dependent Lorentzian component in the noise spectrum (Lindemann & Van Driessche, 1978). We could never observe any indication of a second, Cs^+-induced Lorentzian in the investigated frequency range. However, Fig. 4 showed a decrease in the 'spontaneous' f_c values with increasing Cs^+ concentrations while S_0 also dropped. From the model developed by Lindemann & Van Driessche (1978) it can be predicted that this downwards shift of the 'spontaneous' f_c should originate from a fast blocking mechanism elicited by the Cs^+ ions. The corresponding Cs^+-induced Lorentzian should appear at frequencies much higher than the investigated range and may be masked by the amplifier noise.

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REFERENCES

DeFelice, L. J. (1977). Fluctuation analysis in neurobiology. *Int. Rev. Neurobiol.* **20**, 169–208.

Ehrlich, E. N. & Crabbé, J. (1968). The mechanism of action of amipramizide. *Pflügers Arch.* **302**, 79–96.

Fishman, H. M., Moore, L. E. & Poussart, D. J. M. (1975). Potassium ion conduction noise in squid axon membranes. *J. Membrane Biol.* **24**, 305–328.

Fishman, H. M., Poussart, D. J. M. & Moore, L. E. (1975). Noise measurements in squid axon membrane. *J. Membrane Biol.* **24**, 281–304.

Helman, S. I. & Fisher, R. S. (1977). Microelectrode studies of the active Na transport pathway of frog skin. *J. gen. Physiol.* **69**, 571–604.

Hirschmann, W. & Nagel, W. (1978). The outer membrane of frog skin: impermeable to K^+? *Pflügers Arch.* **373**, R48.

Isenberg, G. (1976). Cardiac Purkinje fibers: Cesium as a tool to block inward rectifying potassium currents. *Pflügers Arch.* **365**, 99–106.

Katz, U. (1978). Changes in ionic conductances and in sensitivity to amiloride during the natural moulting cycle of toad skin (*Bufo viridis*, L.) *J. Membrane Biol.* **38**, 1–9.

Koefoed-Johnsen, V. & Ussing, H. H. (1958). The nature of the frog skin potential. *Acta physiol. scand.* **42**, 298–308.

Leblanc, G. (1972). The mechanism of lithium accumulation in the isolated frog skin epithelium. *Pflügers Arch.* **337**, 1–18.

Lindemann, B. & Van Driessche, W. (1977). Sodium-specific membrane channels of frog skin are pores: Current fluctuations reveal high turnover. *Science, N.Y.* **195**, 292–294.

LINDEMANN, B. & VAN DRIESSCHE, W. (1978). The mechanism of Na-uptake through Na-selective channels in the epithelium of frog skin. In *Membrane Transport Processes*, vol. 1, ed. HOFFMANN, J. F., pp. 155–185. New York: Raven.

MORENO, J. (1974). Blockage of cation permeability across the tight junctions of gallbladder and other leaky epithelia. *Nature, Lond.* **251**, 150–151.

MÜLLER, P. (1965). Potassium and rubidium exchange across the surface membrane of cardiac Purkinje fibres. *J. Physiol.* **177**, 453–462.

NAGEL, W. (1978). Evidence for electrogenic Na transport from the cytoplasmic tissue pool of frog skin epithelium. *J. Physiol.* **284**, 146–147*P*.

NIELSEN, R. (1971). Effect of amphotericin B on the frog skin *in vitro*. Evidence for outward active potassium transport across the epithelium. *Acta physiol. scand.* **83**, 106–114.

SHUM, W. K. & FANELLI, M. JR. (1978). Does intracellular sodium regulate sodium transport across the mucosal surface of frog skin? *Biochim. Biophys. Acta* **512**, 593–597.

ULBRICHT, W. (1977). Ionic channels and gating currents in excitable membranes, *A. Rev. Biophys. Bioeng.* **6**, 7–31.

VAN DRIESSCHE, W. & GÖGELEIN, H. (1978). Potassium channels in the apical membrane of the toad gallbladder. *Nature, Lond.* **275**, 665–667.

VAN DRIESSCHE, W. & LINDEMANN, B. (1978). Low-noise amplification of voltage and current fluctuations arising in epithelia. *Rev. Sci. Instrum.* **49**, 52–7.

VAN DRIESSCHE, W. & ZEISKE, W. (1978). Fluctuations of the K^+-current in the frog skin (*Rana temporaria*). *Archs int. Physiol. Biochim.* **86**, 684–687.

VERVEEN, A. A. & DEFELICE, L. J. (1974). Membrane Noise. In *Progress in Biophysical and Molecular Biology*, **28**, pp. 189–265, ed. BUTLER, A. J. V. & NOBLE, D. Oxford & New York: Pergamon.

ZEISKE, W. (1976). The influence of 2.4.6-triaminopyrimidine on Na^+-transport in frog skin. *Pflügers Arch.* **362**, *R*28.

ZEISKE, W. & VAN DRIESSCHE, W. (1978*a*). K^+-uptake across the outer border of frog skin (*R. temp.*) and its inhibition by Cs-ions. *Pflügers Arch.* **373**, R48.

ZEISKE, W. & VAN DRIESSCHE, W. (1978*b*). The origin of K^+-dependent current fluctuations in frog skin (*R. temp.*). *Pflügers Arch.* **373**, R48.

ZEISKE, W. & VAN DRIESSCHE, W. (1979). A saturable K^+-pathway across the outer border of frog skin (*Rana temporaria*): Kinetics and inhibition by Cs^+ and other cations. *J. Membrane Biol.* **47**, 77–96.