Ca\(^{2+}\)-blockable, Poorly Selective Cation Channels in the Apical Membrane of Amphibian Epithelia

\(UO_2^{2+}\) Reveals Two Channel Types

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**Abstract** This study deals with the effect of mucosal \(UO_2^{2+}\) on the \(Ca^{2+}\)-blockable, poorly selective cation channels in the apical membrane of frog skin and toad urinary bladder. Our data show that \(UO_2^{2+}\) inhibits the \(Na^+\) currents through the amiloride-insensitive cation pathway and confirm a previously described stimulatory effect on the amiloride-blockable \(Na^+\) transport. Noise analysis of the \(Ca^{2+}\)-blockable current demonstrates that the divalent also depresses the low-frequency Lorentzian \((f_c = 11.7\) Hz\) in the power density spectrum (PDS) and reveals the presence of high-frequency relaxation noise \((f_c = 58.5\) Hz\). The action of \(UO_2^{2+}\) is not reversed upon washout and is not accompanied by noise, typically induced by reversible blockers. The divalent merely depresses the plateau of the low-frequency Lorentzian, demonstrating a decrease in the number of conductive cation channels. Similarly, with mucosal \(K^+\) and \(Rb^+\), \(UO_2^{2+}\) also unmasks the high-frequency Lorentzian by depressing the noise from the slowly fluctuating cation channels (type S). In all experiments with mucosal \(Cs^+\), the PDS contains high-frequency relaxation noise \((f_c = 75.1\) Hz in \textit{Rana temporaria}, and 65.4 Hz in \textit{Rana ridibunda}). An effect of \(UO_2^{2+}\) on the \(Cs^+\) currents and Lorentzian plateaus could not be demonstrated, suggesting that this monovalent cation does not pass through type S channels. Experiments with the urinary bladder revealed only a \(UO_2^{2+}\)-insensitive pathway permeable for \(Na^+\), \(K^+\), \(Rb^+\), and \(Cs^+\). We submit that in frog skin two cation-selective channels occur, distinguished by their spontaneous gating kinetics, their sensitivity to \(UO_2^{2+}\), and their permeability for \(Cs^+\). In toad urinary bladder, only one kind of cation-selective channel is observed, which resembles the \(UO_2^{2+}\)-insensitive channel in frog skin, with fast open-closed kinetics (type F).

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INTRODUCTION

Previous studies from our laboratory demonstrated that removal of Ca\textsuperscript{2+} from the mucosal bath reveals the presence of cation-selective channels in the apical membrane of frog skin (Van Driessche and Zeiske, 1985; Van Driessche, Desmedt, and Simaels, 1991) and toad urinary bladder (Aelvoet, Erlij, and Van Driessche, 1988). These channels are amiloride insensitive, but they are blocked by (sub)micromolar concentrations of Ca\textsuperscript{2+}. The concentrations for half-maximal inhibition of the amiloride-insensitive Na\textsuperscript{+} current are \( \sim 200 \) and 450 nM in the skin and bladder, respectively (Van Driessche et al., 1991). Another difference between the channels in both tissues concerns their open-closed kinetics. In frog skin the channel flickering gives rise to a Lorentzian component with corner frequencies \( (f_c) \) of \( \sim 12 \) Hz, whereas in the toad bladder \( f_c \) is 347 Hz (Van Driessche et al., 1991). This variation could result from differences in the channel's environment which modulate its gating and sensitivity to Ca\textsuperscript{2+}. Alternatively, it suggests that more than one channel type exists, a hypothesis dealt with in this article.

In the bladder we extensively studied the permeability for different monovalent cations, whereas in the skin we mainly focused on movements of Na\textsuperscript{+} through this pathway. To unequivocally establish whether or not this amiloride-insensitive Na\textsuperscript{+} transport was (in part) the consequence of an incomplete amiloride action (Cuthbert and Wong, 1972), we performed a detailed study on the interference of mucosal Ca\textsuperscript{2+} with amiloride block (Desmedt, Simaels, and Van Driessche, 1991). The main conclusions from this study were that (a) the inhibitory potency of the diuretic is not affected by external Ca\textsuperscript{2+} per se, and (b) differences in amiloride block observed between tight epithelia (frog skin, toad urinary bladder, A6 cells) can be ascribed to the presence or absence of the cation-selective pathway. These results contradict the hypothesis that divalents, and Ca\textsuperscript{2+} in particular, are required to assure full inhibitory potency of amiloride. One of the divalents reported to serve as a substitute for Ca\textsuperscript{2+} in this respect is UO\textsubscript{2}\textsuperscript{2+} (Benos, Simon, Mandel, and Cala, 1976). In our opinion, this observation most probably reflects interferences between UO\textsubscript{2}\textsuperscript{2+} and the cation-selective pathway. It is the purpose of this report to cover this interaction in detail. The results of this study show that in frog skin, this divalent inhibits mainly the part of the Ca\textsuperscript{2+}-blockable current that is associated with the low-frequency Lorentzian \( (f_c = 12 \) Hz) reported previously (Van Driessche et al., 1991). At the same time, UO\textsubscript{2}\textsuperscript{2+} reveals the presence of a high-frequency Lorentzian component that was masked by the low-frequency noise. The characteristics of the relaxation noise in the toad urinary bladder resemble those of the high-frequency Lorentzian component in frog skin.

MATERIALS AND METHODS

Tissue Preparation

This study was performed with ventral skins of frog species Rana temporaria and Rana ridibunda, and the urinary bladders of Bufo marinus toads. Frogs were kept at 17°C in plastic containers, whereas toads resided at room temperature on wet peat moss. The animals had free access to tap water. The tissues were dissected from doubly pithed animals and glued with the serosal surface to a Lucite ring with cyanoacryl glue (Hystoacryl; B. Braun Melsungen AG, Melsungen,
Germany) and mounted between two Lucite chamber compartments as described before (De Wolf and Van Driessche, 1986). Both the apical and basolateral compartments had a volume of 1.7 ml, and were continuously perfused at a rate of ~7 ml/min. The tissue area in contact with the bathing media was 0.5 cm². The chamber halves were connected to a low-noise voltage clamp through 1 M KCl/agar (3%) bridges, 1 M KCl, and Ag/AgCl wires. The transepithelial potential was clamped to zero, except during the 256-ms voltage steps of 5-mV amplitude, which were applied every 14 s to measure the transepithelial conductance (Gt). Transepithelial currents were continuously recorded on a strip-chart recorder. Short-circuit current (Isc) and Gt were digitized and stored on disk for later analysis. Cation movements from mucosa to serosa are by convention represented as a positive current.

**Noise Analysis**

The fluctuation in current was recorded and analyzed in the frequency domain. Briefly, the current signal was high-pass filtered with a 24-dB/octave Butterworth type filter with a cut-off frequency of 0.3 Hz. Furthermore, to prevent aliasing the signal was low-pass filtered with a 48-dB/octave Butterworth filter (cut-off frequency = 850 Hz). Power density spectra (PDS) were calculated from Fourier transformed records of 4,096 points collected over a 2-s period yielding a fundamental frequency of 0.5 Hz. Averaged PDSs of 2,048 frequencies were obtained from 50 records. Frequencies > 760 Hz were discarded in the further analysis. The number of spectral data points was reduced by calculating the mean of the power densities around frequencies located at equal distances on the logarithmic frequency axis. Two spectra containing 44 and 66 frequency lines were derived with five and eight frequencies per octave, respectively. The 44-line spectrum was used for representation purposes (see figures), whereas the 66-line spectrum was used for numerical analysis. Spectra containing relaxation noise were fitted with the sum of a Lorentzian and an 1/fα term according to the following formula:

\[ S(f) = \frac{S_0}{1 + (f/f_\alpha)^2} + \frac{A}{f^\alpha} \]

The plateau value (S₀) and the corner frequency (f_α) of the Lorentzian function were determined by nonlinear regression analysis.

**Solutions**

Highly purified water (Milli-Q: Water Purification System; Millipore Corp., Bedford, MA) was used to prepare the solutions. The serosal side of skins and bladders was perfused with Na₂SO₄ Ringer's solution, containing (mM): 115 Na⁺, 58.5 SO₄²⁻, 2.5 K⁺, 2.5 HCO₃⁻, and 1 Ca²⁺ (pH 8.2). In some experiments Na⁺ was partially replaced with K⁺ to achieve a final concentration of 37.5 mM K⁺ (Fig. 5 and Table III) or 92.5 mM K⁺ (Fig. 1 B and Table I). Bladders were treated with 0.1 U/ml oxytocin (Sigma Chemical Co., St. Louis, MO) to activate maximally the cation-selective transport (Aelvoet et al., 1988). The mucosal side was bathed with Ca²⁺-free solutions with the following composition (mM): 115 Na⁺, K⁺, Cs⁺, or Rb⁺, 57.5 SO₄²⁻, 5 HEPES, 0.5 EGTA (ethylene glycol-O,O'-bis[2-aminoethyl]amine-N,N',N',N'-tetraacetic acid). pH was adjusted to 7.5 with NaOH, KOH, or Tris. To block the highly specific Na⁺ channels, 10 or 60 µM amiloride (Sigma Chemical Co.) was added to the mucosal solutions containing Na⁺. The Ca²⁺-blockable component of Iₛ (IₛCa²⁺) and the effect of Ca²⁺ on the noise spectra were measured by adding 1.5 mM Ca²⁺ to the EGTA-containing solutions (Fabiato and Fabiato, 1979). UO₂²⁻ was added as nitrate to the mucosal bath in a concentration of nominally 10 or 100 µM. The stoichiometric stability constants for EGTA (Martell and Smith, 1974) indeed indicate that uranyl (UO₂²⁻) complexes with the chelator, and that, under our experimental conditions, only a small fraction of the divalent (<0.01%) is expected to exist as a free cation.
This fraction is calculated from the published equilibrium constant $K_2$ between $\text{UO}_2^{2+}$ and EGTA.H (Martell and Smith, 1974; Fabiato and Fabiato, 1979). However, one must also take into account the occurrence of uranylhydroxy salts or even anionic uranato complexes, especially at basic pH (Katz and Seaborg, 1957). Consequently, it seems very cumbersome to determine the exact concentrations of free $\text{UO}_2^{2+}$ and its complexes. For the purpose of this study, solving these complications was rather irrelevant, and we therefore contented ourselves with using the concentration of $\text{UO}_2^{2+}$ in a purely operational sense. Also, no attempt is made to determine which forms of $\text{UO}_2^{2+}$ interfere with the cation channels.

RESULTS

Stimulatory Effect of Mucosal $\text{UO}_2^{2+}$ on the Amiloride-blockable $\text{Na}^+$ Transport

In the first series of experiments, we tested the influence of 100 μM mucosal $\text{UO}_2^{2+}$ on the amiloride-blockable $\text{Na}^+$ channel in the presence and absence of mucosal $\text{Ca}^{2+}$ in skins of $R. \text{temporaria}$. Experiments in the presence of $\text{Ca}^{2+}$ were performed with tissues exposed to $\text{Na}_2\text{SO}_4$ solutions on both sides. To investigate the effect of $\text{UO}_2^{2+}$ in $\text{Ca}^{2+}$-free conditions we depolarized the basolateral membranes with high $\text{K}^+$-containing solutions (92.5 mM $\text{K}^+$). We used this concentration because it completely abolishes the net current through the $\text{Ca}^{2+}$-blockable, cation-selective pathway in the apical membrane in short-circuited conditions (Desmedt et al., 1991). Consequently, in depolarized skins exposed to $\text{Ca}^{2+}$-free mucosal solutions, changes in $I_{sc}$ elicited by $\text{UO}_2^{2+}$ are expected to reflect effects on the amiloride-blockable $\text{Na}^+$ channels. It must be noted that the $\text{Ca}^{2+}$ chelator EGTA was always present, in the $\text{Ca}^{2+}$-containing as well as in the $\text{Ca}^{2+}$-free mucosal Ringer's solution. In this way, comparison of the results obtained in different conditions is not biased by any effects due to the chelator itself.

Fig. 1A exemplifies an experiment with a nondepolarized skin. The addition of $\text{UO}_2^{2+}$ induced an increase of $I_{sc}$ from 22.0 to 27.5 μA/cm². Both the control and the stimulated current were amiloride blockable (10 μM), indicating an effect of $\text{UO}_2^{2+}$ on the highly selective $\text{Na}^+$ channels. Fig. 1B represents a similar experiment with a depolarized tissue. Again $I_{sc}$ increased markedly with mucosal $\text{UO}_2^{2+}$, in this case from 23.2 to 34.8 μA/cm². As in nondepolarized skins, $\text{UO}_2^{2+}$ acted on the $\text{Na}^+$ channel specifically since the current stimulated by $\text{UO}_2^{2+}$ was amiloride blockable (10 μM) as well. Mean values in Table I demonstrate that $\text{UO}_2^{2+}$ elicits a significant ($P < 0.01$) increase of $I_{sc}$ in the depolarized as well as the nondepolarized skins. From the result obtained with depolarized skins, it is furthermore obvious that the removal of mucosal $\text{Ca}^{2+}$ does not affect the inhibitory potency of amiloride. So far, our data indicate that $\text{UO}_2^{2+}$ displays a specific stimulatory effect on the amiloride-blockable $\text{Na}^+$ channel.

Inhibitory Effect of Mucosal $\text{UO}_2^{2+}$ on $\text{Na}^+$ Currents through the Poorly Selective, $\text{Ca}^{2+}$-blockable Pathway

The influence of mucosal $\text{UO}_2^{2+}$ on $\text{Na}^+$ transport through the poorly selective cation pathway was investigated in nondepolarized skins exposed to $\text{Ca}^{2+}$-free mucosal $\text{Na}_2\text{SO}_4$ Ringer's solution containing amiloride. To avoid contaminations of our noise spectra with amiloride-induced noise, we increased the concentration of the diuretic
Figure 1. Effect of UO$_2^{2+}$ (100 μM) on amiloride-blockable Na$^+$ currents through frog skin (R. temporaria). The amiloride-blockable current was measured by adding 10 μM of the diuretic to the mucosal Na$_2$SO$_4$ solution. (A) Recording of I$_{sc}$ in nondepolarized skin exposed to mucosal Ca$^{2+}$-containing Na$_2$SO$_4$ solution. (B) Ca$^{2+}$ was removed from the mucosal bath and the basolateral membranes were K$^+$ depolarized (92.5 mM K$^+$). Here, as well as in the following figures, the vertical current deflections are caused by voltage pulses of 10 mV.

In the experiment depicted in Fig. 1A, the amiloride-insensitive I$_{sc}$ amounted to 9.8 μA/cm$^2$. UO$_2^{2+}$ had a different effect on I$_{sc}$ as compared with the response shown in Fig. 1. The amiloride-insensitive current, instead of being stimulated, was reduced to 2.0 μA/cm$^2$. The remaining current component was further depressed to 0.9 μA/cm$^2$ with Ca$^{2+}$. The washout of UO$_2^{2+}$ did not restore I$_{sc}$ (unpublished observation). Mean values in Table II demonstrate a significant ($P < 0.01$) inhibition of the amiloride-insensitive I$_{sc}$ by UO$_2^{2+}$.

The most interesting finding so far is that UO$_2^{2+}$ is able to inhibit Na$^+$ currents through the poorly selective cation pathway, whereas it stimulates Na$^+$ movements through the amiloride-blockable channels. The latter process has been reported to be reversible (Zeiske, 1978), whereas our results on the Ca$^{2+}$-blockable pathway demon-

Table 1

|                       | Nondepolarized | Depolarized |
|-----------------------|----------------|-------------|
| Control               | 16.5 ± 1.8     | 20.3 ± 3.7  |
| Amiloride             | 1.7 ± 0.8*     | 0.5 ± 1.7*  |
| UO$_2^{2+}$           | 23.2 ± 1.9*    | 31.7 ± 5.8* |
| UO$_2^{2+}$ + amiloride| 1.9 ± 0.9*     | 0.9 ± 1.4*  |

$I_{sc}$ (μA/cm$^2$) values recorded while skins (R. temporaria) were serosally perfused with either Na$_2$SO$_4$ (nondepolarized) or K$_2$SO$_4$ (92.5 mM K$^+$ depolarized). The mucosal solution was Na$_2$SO$_4$ with or without Ca$^{2+}$ for nondepolarized or depolarized tissues, respectively. Amiloride concentration, 10 μM; UO$_2^{2+}$ concentration, 100 μM. n = 5. All values are given as means ± SEM.

*Values significantly different from control at $P < 0.05$.

*Values significantly different from the preceding experimental conditions at $P < 0.05$. 
FIGURE 2. Inhibition of \( I_{\text{sc}}^{\text{Ca}} \) and the low-frequency Lorentzian noise by 100 \( \mu \text{M} \) mucosal UO\(_2^{2+}\). The skin of \( R. \text{temporaria} \) was bilaterally exposed to \( \text{Na}_2\text{SO}_4 \) solutions. The mucosal bath contained 60 \( \mu \text{M} \) amiloride. 1.5 mM Ca\(^{2+}\) was added to the EGTA-containing mucosal solution to measure \( I_{\text{sc}}^{\text{Ca}} \). PDSs in \( B \) were recorded at the time intervals marked at the current trace in \( A \). UO\(_2^{2+}\) depresses the low-frequency Lorentzian and unmasks a high-frequency Lorentzian.

strate an irreversible effect. The data in Table II demonstrate that 1 mM Ca\(^{2+}\) exerts an additional inhibitory effect in the presence of 100 \( \mu \text{M} \) UO\(_2^{2+}\), which might indicate that this dose is too low. Higher amounts of UO\(_2^{2+}\) (up to 1 mM) do indeed reduce the Ca\(^{2+}\)-blockable \( I_{\text{sc}} \) further (unpublished observation). However, the results from noise analysis of the UO\(_2^{2+}\) block, described below, suggest that the inhibition obtained with higher doses occurs through occlusion of another pathway: up to 100 \( \mu \text{M} \), UO\(_2^{2+}\) has a specific inhibitory interaction with only part of the cation-selective channels, whereas concentrations near 1 mM affect all channels, thereby distinguishing between two channel types.

In previous work (Van Driessche et al., 1991), we established that Ca\(^{2+}\) removal from the mucosal bath markedly raises noise levels. A Lorentzian component with \( f_c \) values in the range of 10–20 Hz was frequently present in the PDS. In this article we will refer to these Lorentzians as low-frequency noise components. The example

| Table II |
| --- |
| Effect of UO\(_2^{2+}\) on the Poorly Selective Cation Pathway |
| Control | 15.5 \( \pm \) 2.9 |
| Amiloride | 7.6 \( \pm \) 1.6* |
| Amiloride + UO\(_2^{2+}\) | 3.4 \( \pm \) 0.8^1 |
| Amiloride + UO\(_2^{2+}\) + Ca\(^{2+}\) | 0.3 \( \pm \) 0.3 |

\( I_{\text{sc}} \) (\( \mu \text{A/cm}^2 \)) values were recorded in nondepolarized skins (\( R. \text{temporaria} \)) bathed with Ca\(^{2+}\)-free \( \text{Na}_2\text{SO}_4 \) solution on the mucosal side. Amiloride concentration, 60 \( \mu \text{M} \); UO\(_2^{2+}\) concentration, 100 \( \mu \text{M} \). \( n = 6 \).

All values are given as means \( \pm \) SEM.

*Values significantly different from control at \( P < 0.05 \).

^1Values significantly different from the preceding experimental conditions at \( P < 0.05 \).
demonstrated in Fig. 2B originates from the same experiment as the \( I_{sc} \) recording shown in Fig. 2A. The control spectrum contains a Lorentzian component with \( f_c = 11.4 \text{ Hz} \) and \( S_o = 641 \times 10^{-21} \text{ A}^2\text{s/cm}^2 \), which is irreversibly depressed by 100 \( \mu \text{M} \) \( \text{UO}_2^{2+} \). Concomitantly, another relaxation noise component appears at higher frequencies in the PDS. For the experiment shown, the PDS recorded with \( \text{UO}_2^{2+} \) was fitted with the Lorentzian parameters \( f_c = 78.4 \text{ Hz} \) and \( S_o = 20.4 \times 10^{-21} \text{ A}^2\text{s/cm}^2 \). Subsequent administration of \( \text{Ca}^{2+} \) abolishes this noise component. The appearance of the high-frequency component after \( \text{UO}_2^{2+} \) is in striking contrast to the complete inhibitory effect of \( \text{Ca}^{2+} \) (Fig. 2B, and Van Driessche et al., 1991). Means of \( f_c \) and \( S_o \) together with the corresponding \( I_{sc}^0 \) values are listed in Table III (low-frequency component \([2.5 \text{ K}^+])\). They convey the idea that \( \text{UO}_2^{2+} \) inhibits a fluctuating channel associated with the low-frequency Lorentzian noise (channel type S) reported previously (Van Driessche et al., 1991), and concomitantly reveals the presence of another channel type that generates a high-frequency Lorentzian noise component (channel type F).

**Table III**

|                      | \( n \) | \( f_c \)     | \( S_o \times 10^{21} \) | \( I_{sc}^0 \) | \( \mu\text{A/cm}^2 \) |
|----------------------|--------|---------------|--------------------------|--------------|---------------------|
| **Low-frequency component \([2.5 \text{ K}^+]\)** |        |               |                          |              |                     |
| Control              | 6      | 11.7 ± 1.0    | 542.5 ± 139.9            | 14.2 ± 7.2   |                     |
| 10 \( \mu \text{M} \) \( \text{UO}_2^{2+} \) | 6      | --            | --                       | --           |                     |
| 100 \( \mu \text{M} \) \( \text{UO}_2^{2+} \) | 6      | 58.5 ± 4.6*   | 27.4 ± 6.2*              | 2.3 ± 0.5*   |                     |
| **Low-frequency component \([37.5 \text{ K}^+]\)** |        |               |                          |              |                     |
| Control              | 5      | 16.0 ± 1.7    | 121.5 ± 34.9             | 1.8 ± 0.5    |                     |
| 10 \( \mu \text{M} \) \( \text{UO}_2^{2+} \) | 4      | 17.6 ± 1.2    | 26.5 ± 12.6*             | 0.4 ± 0.1*   |                     |
| 100 \( \mu \text{M} \) \( \text{UO}_2^{2+} \) | 5      | 16.6 ± 2.6    | 6.0 ± 2.3*               | 0.2 ± 0.0*   |                     |
| **High-frequency component \([2.5 \text{ K}^+]\)** |        |               |                          |              |                     |
| Control              | 10     | 100.4 ± 6.9   | 49.0 ± 7.5               | 10.2 ± 1.6   |                     |
| 10 \( \mu \text{M} \) \( \text{UO}_2^{2+} \) | 5      | 98.7 ± 12.6   | 45.1 ± 8.3               | 4.3 ± 0.8*   |                     |
| 100 \( \mu \text{M} \) \( \text{UO}_2^{2+} \) | 10     | 96.4 ± 7.7    | 32.5 ± 4.4*              | 2.5 ± 0.4*   |                     |

Experiments with skins of *R. temporaria* exposed to \( \text{Ca}^{2+}\)-free \( \text{Na}_2\text{SO}_4 \) solution mucosally. In the category marked “low-frequency component \([37.5 \text{ K}^+]\)”, 100 \( \mu \text{M} \) tetracaine was added to the mucosal bathing solution (see text).

All values are given as means ± SEM.

*Values significantly different from control at \( P < 0.05 \).

Evidence for Two Different \( \text{Ca}^{2+}\)-blockable Channel Types

As a matter of fact, whereas some tissues display low-frequency Lorentzians, others provide high-frequency Lorentzian components in the absence of mucosal \( \text{UO}_2^{2+} \). An example is depicted in Fig. 3. The control spectrum in Fig. 3B consists of substantial 1/f noise in the lower frequency part, and a relaxation noise at the higher frequency end with \( f_c = 135.1 \text{ Hz} \) and \( S_o = 56.6 \times 10^{-21} \text{ A}^2\text{s/cm}^2 \). The addition of 10 \( \mu \text{M} \)
UO$_2^{2+}$ in this case reduces the 1/f noise, but leaves the Lorentzian component almost unaltered. Further elevation of the UO$_2^{2+}$ concentration to 100 μM does not change the PDS. Finally, the addition of Ca$^{2+}$ to the mucosal side completely abolishes the relaxation noise, and depresses 1/f noise further. Basically, this experiment demonstrates that the influence of UO$_2^{2+}$ on the spontaneous high-frequency Lorentzian is rather limited. $I_{sc}$, on the other hand, is markedly reduced with 10 μM UO$_2^{2+}$ (Fig. 3 A). The elevation of the UO$_2^{2+}$ concentration to 100 μM merely results in a slight additional inhibition of $I_{sc}$ only. Mean values obtained from experiments with this kind of skins (high-frequency component data in Table III) confirm that 10 μM UO$_2^{2+}$ significantly (P < 0.05) decreases $I_{sc}^{ca}$ without altering $S_o$ or $f_c$ (P > 0.25). The increase of the dose to 100 μM has a significant (P < 0.01), but relatively small (34%), inhibitory effect on the $S_o$ of the high-frequency Lorentzian (compared with the 86% inhibition of the low-frequency Lorentzian in Fig. 5 B). The apparent discrepancy between the effect of UO$_2^{2+}$ on $I_{sc}$ and $S_o$ can be understood if one assumes that the 1/f noise observed in the control spectrum of Fig. 3 B is at least partly related to currents passing through the UO$_2^{2+}$-blockable channels described before. The observation that the depression of the 1/f noise by 10 μM UO$_2^{2+}$ is accompanied by a marked reduction of $I_{sc}$ favors this assumption. Additional support comes from experiments with tissues where 1/f noise is not expressed, or only poorly expressed. In these tissues the high-frequency Lorentzians (Fig. 4 B), as well as $I_{sc}^{ca}$ (Fig. 4 A), are insensitive to UO$_2^{2+}$. These findings clearly demonstrate that cation-selective channels with fast open-closed kinetics (type F) are not blocked by 100 μM UO$_2^{2+}$. The data also show that the lack of UO$_2^{2+}$-inhibitable 1/f noise is correlated with the absence of UO$_2^{2+}$-blockable currents.

**Figure 3.** Effect of 10 and 100 μM UO$_2^{2+}$ on $I_{sc}^{ca}$ and the high-frequency Lorentzian noise component. As in Fig. 2, we used the skins of *R. temporaria* perfused with Na$_2$SO$_4$ on both sides. Amiloride was present in the mucosal bath. The PDSs in B were recorded during the experiment shown in A at time intervals marked with numbers at the current trace.
UO$_2^{2+}$ Blocks the Slowly Gated Process and Does Not Induce Blocker Noise

In many experimental protocols, the presence of a 1/f noise component considerably hampers the study of the Lorentzian noise associated with currents passing through the Ca$^{2+}$-blockable pathway. For example, the extent to which the low-frequency Lorentzian is depressed with UO$_2^{2+}$ cannot be assessed because quite early in the course of this inhibitory process the low-frequency Lorentzian is masked by 1/f noise. Ideally, the investigation of direct effects of UO$_2^{2+}$ on the low-frequency Lorentzian requires low levels of 1/f noise. We found that a moderate elevation of serosal K$^+$...
(37.5 mM) diminishes the 1/f amplitudes and unmasks the low-frequency Lorentzian noise (Fig. 5 B). A high-frequency Lorentzian with $f_c = 93.9$ Hz and $S_o = 18.9 \times 10^{-21}$ A$^2$ s/cm$^2$ is still detectable above the otherwise substantial background noise levels. To investigate the effects on the low-frequency Lorentzian only, we depressed the high-frequency relaxation noise by adding 100 µM tetracaine to the mucosal bath. For the moment, the comparison of spectra 1 and 2 in Fig. 5 B should suffice to convince the reader, but in the companion paper (Desmedt, Simaels, and Van Driessche, 1993) we will demonstrate in more detail that this concentration of the local anesthetic indeed selectively abolishes the high-frequency noise component. The subsequent replacement of 35 mM Na$^+$ in the serosal bath by K$^+$ depressed the 1/f noise and revealed the presence of a low-frequency Lorentzian with $f_c = 10.4$ Hz and $S_o = 127 \times 10^{-21}$ A$^2$ s/cm$^2$. The mean values for both $S_o$ and $f_c^{2+}$ are much lower than in 2.5 serosal K$^+$ (Table III), which is probably due to a diminished electrical driving force caused by the K$^+$ depolarization. The addition of 100 µM UO$_2^{2+}$ further inhibited $f_c^{2+}$, and the time-dependent changes in the Lorentzian noise could now be quantified. Clearly, the plateau value of the low-frequency Lorentzian was lowered with UO$_2^{2+}$ to $17.4 \times 10^{-21}$ A$^2$ s/cm$^2$ or less, but $f_c$ essentially remained constant. Table III lists the mean values for $f_c^{2+}$, $f_o^{2+}$, and $S_o$ in control, with 10 and 100 µM UO$_2^{2+}$. This experiment shows that UO$_2^{2+}$ merely depresses $S_o$ without inducing additional current fluctuation or shifting the $f_c$. Therefore, the high-frequency component unmasked by UO$_2^{2+}$ (Fig. 2 B) cannot be identified with UO$_2^{2+}$-induced blocker noise. Taken together with the irreversible character of the UO$_2^{2+}$ effect (see above), this result strongly suggests that UO$_2^{2+}$ exerts its blocking action on the low-frequency component by a reduction in the number of conductive units, caused by an irreversible occlusion of the channel pores, and not by changing the open probability of the channels.

![Figure 6](https://example.com/fig6.png)

**Figure 6.** Effect of 100 µM UO$_2^{2+}$ on the Ca$^{2+}$-blockable K$^+$ current and noise in *R. ridibunda*. Tissues were perfused with Ca$^{2+}$-free K$_2$SO$_4$ and regular Na$_2$SO$_4$ solution on the mucosal and serosal sides, respectively. UO$_2^{2+}$ depresses $f_c^{2+}$ and the low-frequency Lorentzian noise. As in Fig. 2 B, the high-frequency relaxation noise is unmasked by UO$_2^{2+}$. 

- **A**
  - Ca$^{2+}$
  - UO$_2^{2+}$
  - $I_{sc}$
  - µA/cm$^2$
  - Time (min)

- **B**
  - $S(f)$
  - $K^2$/s/cm$^2$
  - f (Hz)

Table III lists the mean values for $I_{sc}$, $f_o$, and $S_o$ in control, with 10 and 100 µM UO$_2^{2+}$. This experiment shows that UO$_2^{2+}$ merely depresses $S_o$ without inducing additional current fluctuation or shifting the $f_c$. Therefore, the high-frequency component unmasked by UO$_2^{2+}$ (Fig. 2 B) cannot be identified with UO$_2^{2+}$-induced blocker noise. Taken together with the irreversible character of the UO$_2^{2+}$ effect (see above), this result strongly suggests that UO$_2^{2+}$ exerts its blocking action on the low-frequency component by a reduction in the number of conductive units, caused by an irreversible occlusion of the channel pores, and not by changing the open probability of the channels.
Effect of $\text{UO}_2^{2+}$ on $K^+$ Currents through the Poorly Selective $\text{Ca}^{2+}$-blockable Channels

To further substantiate the inhibitory action of $\text{UO}_2^{2+}$ on the poorly selective cation pathway and the separation of this latter into two components, we tested its effect on other cationic currents passing through the $\text{Ca}^{2+}$-blockable pathways. The experiments with $K^+$ as the main mucosal cation were performed on skins of $R$. ridibunda. In this frog species the cation-selective pathway is also expressed, whereas $K^+$ channels as found in the apical membrane of the skin of $R$. temporaria (De Wolf and Van Driessche, 1986) are usually not detectable with noise analysis. Consequently, $\text{Ca}^{2+}$-blockable noise components recorded with $K^+$ are not expected to be seriously contaminated by the presence of the $\text{Ca}^{2+}$-insensitive $K^+$ channels. In the experiment shown in Fig. 6, the $\text{Ca}^{2+}$-blockable $K^+$ current through the cation-selective pathway amounted to 16.9 $\mu$A/cm$^2$ and yielded a spontaneous Lorentzian noise component with $f_e = 30.5$ Hz and $S_o = 1,990 \times 10^{-21}$ A$^2$/s/cm$^2$ in control conditions. After administration of $\text{UO}_2^{2+}$, $I^C_{\text{Ca}}$ reached a steady-state value of 3.4 $\mu$A/cm$^2$. This experiment also demonstrates that $\text{UO}_2^{2+}$ exerts its inhibitory effect most obviously independent of amiloride since the diuretic was absent (see Discussion). Concomitantly, the low-frequency Lorentzian was gradually depressed by $\text{UO}_2^{2+}$, revealing another Lorentzian with higher $f_e$ ($f_e = 166.0$ Hz; $S_o = 66.5 \times 10^{-21}$ A$^2$/s/cm$^2$), which was readily inhibitable with $\text{Ca}^{2+}$. Therefore, also with $K^+$, the use of $\text{UO}_2^{2+}$ seemed to indicate and distinguish between two different gating processes. Means of $I^C_{\text{Ca}}$ and the Lorentzian parameters are listed in Table IV. A few additional experiments with Rb$^+$ as the main mucosal cation yielded similar results (unpublished observation).

### Table IV

| Conditions | $n$ | $f_e$ (Hz) | $S_o \times 10^{21}$ (A$^2$/s/cm$^2$) | $I^C_{\text{Ca}}$ (\mu A/cm$^2$) |
|------------|-----|-----------|-----------------------------------|----------------------------------|
| $K^+ \ (R$. ridibunda$)$ | | | | |
| Control    | 5   | 27.6 ± 1.5 | 1045.7 ± 437.0                     | 9.3 ± 2.4                        |
| $\text{UO}_2^{2+}$ | 5   | 122.3 ± 14.6* | 35.8 ± 9.3*                      | 2.1 ± 0.6*                       |
| $K^+$ (toad urinary bladder) | | | | |
| Control    | 4   | 406.5 ± 8.3 | 13.0 ± 3.0                        | 9.3 ± 1.8                        |
| $\text{UO}_2^{2+}$ | 4   | 441.3 ± 17.2 | 18.6 ± 4.4                        | 7.2 ± 1.4                        |
| $\text{Cs}^+(R$. ridibunda$)$ | | | | |
| Control    | 5   | 65.4 ± 4.3  | 50.5 ± 6.7                        | 3.5 ± 0.6                        |
| $\text{UO}_2^{2+}$ | 5   | 82.9 ± 8.1  | 28.3 ± 2.3*                      | 2.1 ± 0.4*                       |
| $\text{Cs}^+(R$. temporaria$)$ | | | | |
| Control    | 4   | 75.1 ± 4.3  | 39.9 ± 6.2                        | 1.7 ± 0.2                        |
| $\text{UO}_2^{2+}$ | 4   | 86.9 ± 9.3  | 25.7 ± 3.4*                      | 1.0 ± 0.2*                       |

$\text{UO}_2^{2+}$ concentration, 100 $\mu$M.

All values are given as means ± SEM.

*Values significantly different from control at $P < 0.05$. 

with $f_e = 30.5$ Hz and $S_o = 1,990 \times 10^{-21}$ A$^2$/s/cm$^2$ in control conditions. After administration of $\text{UO}_2^{2+}$, $I^C_{\text{Ca}}$ reached a steady-state value of 3.4 $\mu$A/cm$^2$. This experiment also demonstrates that $\text{UO}_2^{2+}$ exerts its inhibitory effect most obviously independent of amiloride since the diuretic was absent (see Discussion). Concomitantly, the low-frequency Lorentzian was gradually depressed by $\text{UO}_2^{2+}$, revealing another Lorentzian with higher $f_e$ ($f_e = 166.0$ Hz; $S_o = 66.5 \times 10^{-21}$ A$^2$/s/cm$^2$), which was readily inhibitable with $\text{Ca}^{2+}$. Therefore, also with $K^+$, the use of $\text{UO}_2^{2+}$ seemed to indicate and distinguish between two different gating processes. Means of $I^C_{\text{Ca}}$ and the Lorentzian parameters are listed in Table IV. A few additional experiments with Rb$^+$ as the main mucosal cation yielded similar results (unpublished observation).
Finally, we investigated the permeability of the cation-selective pathway for Cs⁺ in both *R. ridibunda* and *R. temporaria*. The most remarkable finding was that Cs⁺ currents through the cation-selective channels generate relaxation noise components in the high-frequency range of the PDS (e.g., Fig. 4 B), whereas the low-frequency Lorentzian was never observed. An example for *R. ridibunda* is shown in Fig. 7 (f_c = 68.4 Hz; S_o = 36.1 × 10⁻²¹ A² s/cm²; I_{sc}^{Ca} = 2.2 μA/cm²). We recorded the effect of UO₂⁺ for ~60 min. During this time period I_{sc}^{Ca} diminished very slowly to 1.2 μA/cm². This decrease occurred spontaneously, since it was also observed when skins were not exposed to UO₂⁺ (unpublished observation). Moreover, the I_{sc} trace in Fig. 7 A is apparently not altered by the addition of UO₂⁺. Means in Table IV were calculated from values recorded at 54 ± 4 min after administration of UO₂⁺. The
Lorentzian noise also generally underwent gradual changes. In the illustrated experiment, $f_c$ varied from 68.4 to 70.3 Hz, while $S_o$ decreased from 36.1 to $23.5 \times 10^{-21} \text{A}^2 \text{s/cm}^2$. Spontaneous changes in tissues not subjected to $\text{UO}_2^{2+}$ were of comparable magnitude (unpublished observation). Means of the noise parameters and $I_{sc}^{Ca}$ are summarized in Table IV. Although an influence of $\text{UO}_2^{2+}$ on the gating kinetics cannot be denied, it is clear that the divalent brings about only a minor modification as compared with the effect on the low-frequency gating process observed with $\text{Na}^+$, $\text{K}^+$, and $\text{Rb}^+$.

The failure to observe low-frequency Lorentzian noise with $\text{Cs}^+$ strongly suggests that type S channels are not permeable for this cation. This idea is further corroborated by the type of experiment shown in Fig. 8. Initially, the mucosal side was perfused with $\text{Ca}^{2+}$-free $\text{Na}^+$ solution. Under these conditions, $I_{sc}$ amounted to 3.6 $\mu\text{A/cm}^2$ (Fig. 8A). Upon substitution of $\text{Cs}^+$ by $\text{Na}^+$, $I_{sc}^{Ca}$ decreased to 0.8 $\mu\text{A/cm}^2$. Concomitantly, the low-frequency Lorentzian was depressed and a high-frequency Lorentzian appeared in the PDS ($r = 67.1 \text{Hz}$, $S_o = 12.2 \times 10^{-21} \text{A}^2 \text{s/cm}^2$). Mean values ($n = 5$) of $I_{sc}^{Ca}$, $f_c$, and $S_o$ in $\text{Na}^+$ solutions were $6.9 \pm 1.9 \mu\text{A/cm}^2$, $16.2 \pm 1.0 \text{Hz}$, and $555 \pm 163 \times 10^{-21} \text{A}^2 \text{s/cm}^2$, respectively. With mucosal $\text{Cs}^+$, $I_{sc}^{Ca}$, $f_c$, and $S_o$ amounted to $2.2 \pm 0.7 \mu\text{A/cm}^2$, $75.7 \pm 4.3 \text{Hz}$, and $38.3 \pm 11.1 \times 10^{-21} \text{A}^2 \text{s/cm}^2$, respectively.

**Figure 9.** Effect of 100 $\mu\text{M} \text{UO}_2^{2+}$ on the $\text{Ca}^{2+}$-blockable $\text{K}^+$ current and noise in toad urinary bladder. Mucosal solution: $\text{Ca}^{2+}$-free $\text{K}_2\text{SO}_4$; serosal solution: $\text{Na}_2\text{SO}_4$ + 0.1 U/ml oxytocin.

Lorentzian with $f_c = 12.5 \text{Hz}$ and $S_o = 181 \times 10^{-21} \text{A}^2 \text{s/cm}^2$ (Fig. 8B). Upon substitution of $\text{Cs}^+$ by $\text{Na}^+$, $I_{sc}^{Ca}$ decreased to 0.8 $\mu\text{A/cm}^2$. Concomitantly, the low-frequency Lorentzian was depressed and a high-frequency Lorentzian appeared in the PDS ($f_c = 67.1 \text{Hz}$, $S_o = 12.2 \times 10^{-21} \text{A}^2 \text{s/cm}^2$). Mean values ($n = 5$) of $I_{sc}^{Ca}$, $f_c$, and $S_o$ in $\text{Na}^+$ solutions were $6.9 \pm 1.9 \mu\text{A/cm}^2$, $16.2 \pm 1.0 \text{Hz}$, and $555 \pm 163 \times 10^{-21} \text{A}^2 \text{s/cm}^2$, respectively. With mucosal $\text{Cs}^+$, $I_{sc}^{Ca}$, $f_c$, and $S_o$ amounted to $2.2 \pm 0.7 \mu\text{A/cm}^2$, $75.7 \pm 4.3 \text{Hz}$, and $38.3 \pm 11.1 \times 10^{-21} \text{A}^2 \text{s/cm}^2$, respectively.

**Ca$^{2+}$-blockable Currents and Noise in Toad Urinary Bladder Are UO$^2_2$+ Insensitive**

The poorly selective cation pathway in toad urinary bladder provides another system to study the effect of $\text{UO}_2^{2+}$ (Fig. 9). In this series of experiments bladders were
bathed with Ca$^{2+}$-free K$_2$SO$_4$ Ringer's solution at the mucosal side and with Na$_2$SO$_4$ Ringer's solution at the serosal side. Effects of UO$_2^{2+}$ were studied on K$^+$ currents, because previous studies (Aelvoet et al., 1988) demonstrated that the Lorentzian noise was generally larger with mucosal K$^+$ than with Na$^+$ or Cs$^+$. K$^+$ currents and noise were stimulated by 0.1 U/ml oxytocin added to the serosal bath. This resulted in control values for $I_w$, $f_c$, and $S_o$ of 7.5 µA/cm$^2$, 420.6 Hz, and 10.9 × 10$^{-21}$ A$^2$s/cm$^2$, respectively. About 1 h after the addition of 100 µM UO$_2^{2+}$, $I_w$ reached a value of 5.9 µA/cm$^2$, while $f_c$ equaled 450.9 Hz and $S_o$ became 12.0 × 10$^{-21}$ A$^2$s/cm$^2$. Mucosal Ca$^{2+}$ reduced $I_w$ to 1.5 µA/cm$^2$ and completely abolished the Lorentzian noise. Means of currents and noise data are included in Table IV.

**DISCUSSION**

The incentive for these experiments with UO$_2^{2+}$ has come from the observation by Benos et al. (1976) that this divalent cation effectively substitutes for mucosal Ca$^{2+}$ in restoring the complete inhibition of Na$^+$ transport by amiloride. On the other hand, a noise analysis study (Desmedt et al., 1991) on the interference between Ca$^{2+}$ and amiloride revealed that the molecular interaction of the diuretic with the highly specific Na$^+$ channels is not affected by external Ca$^{2+}$ at all. We ascribed the apparent reduced inhibitory potency, as inferred from $I_w$ measurements, to the presence of conductive cation-selective channels in parallel with the highly specific Na$^+$ channels. We further reasoned that the result with UO$_2^{2+}$ obtained by Benos and co-workers (1976) therefore probably reflected an interaction of UO$_2^{2+}$ with the Ca$^{2+}$-blockable pathway.

**Inhibitory Effect of UO$_2^{2+}$ on Ca$^{2+}$-blockable Na$^+$ Currents**

The crucial finding in this study is the inhibition of Na$^+$ transport by UO$_2^{2+}$ in the absence of Ca$^{2+}$ and in the presence of amiloride (Fig. 2A). On the basis of our previous work, however (Desmedt et al., 1991), the hypothesis of an improved amiloride block by UO$_2^{2+}$ in the absence of Ca$^{2+}$ must be rejected. Moreover, an inhibition of $I_w$ with UO$_2^{2+}$ is also observed in the absence of amiloride in frog skins exposed to Ca$^{2+}$-free mucosal K$_2$SO$_4$ Ringer's solution (Fig. 6A). This demonstrates that it is quite unnecessary to invoke an involvement of amiloride in order to understand the inhibitory effect of UO$_2^{2+}$ on Na$^+$ transport. The obvious conclusion instead is that the cation-selective channels themselves are blocked by UO$_2^{2+}$. This is a rather interesting finding in view of the stimulatory action of the divalent on the Na$^+$ channels (Zeiske, 1978; Benos, Latorre, and Reyes, 1981). It is important, then, to know whether or not the interaction site for UO$_2^{2+}$ is closely associated with the channel proteins, since it is equally conceivable that the divalent exerts its effect on the cation-selective channels indirectly. More specifically, whereas the study of the cation-selective channel entails the use of Ca$^{2+}$-free mucosal Ringer's solutions containing a Ca$^{2+}$ chelator (EGTA), the Na$^+$ channel has usually been studied in Ca$^{2+}$-containing mucosal Na$^+$ Ringer's solution lacking a Ca$^{2+}$-chelating agent. Consequently, the observed inhibitory behavior could possibly arise from (a) the
The chelator itself influencing membrane structure and/or intracellular fluid composition; (b) the interaction of the chelator with UO$_2^{2+}$; or (c) the absence of external Ca$^{2+}$ itself changing membrane structure and leading, for instance, to changes in intracellular Ca$^{2+}$. For this reason, we looked at the effect of UO$_2^{2+}$ on the Na$^+$ channel with special emphasis on the composition of the bathing solutions. The data (Fig. 1, Table I) show that, as far as the Na$^+$ channel is concerned, the presence of EGTA per se, its chelating effect on UO$_2^{2+}$, the absence of Ca$^{2+}$ per se, and, finally, the depolarized state of the tissue do not convert a stimulatory behavior into an inhibitory one. Thus, changes in the experimental conditions alone, without an essential involvement of the channels themselves, probably do not suffice to explain the different response observed for the amiloride-blockable Na$^+$ pathway and the Ca$^{2+}$-blockable pathway. It must be mentioned that an inhibitory effect of UO$_2^{2+}$ on active Na$^+$ transport in frog skin has also been reported (Schwartz and Flamenbaum, 1976; Benos et al., 1981). However, close inspection of the bathing solutions utilized by these groups (Schwartz and Flamenbaum, 1976; Zeiske, 1978; Benos et al., 1981) reveals that stimulatory behavior is observed with the SO$_4^{2-}$ anion, whereas the presence of Cl$^-$ leads to inhibitory behavior of UO$_2^{2+}$. With respect to the Ca$^{2+}$-blockable pathway, no such effect was observed. UO$_2^{2+}$ always displays inhibitory behavior on this pathway.

**UO$_2^{2+}$ Does Not Induce Blocker Noise of the Type S Channels**

More convincing arguments in favor of a direct interaction between UO$_2^{2+}$ and the cation-selective channel proteins come from the noise analysis data. In previous work we showed that the presence of the cation-selective channels was often, though not always, associated with the detection of a spontaneous Ca$^{2+}$-blockable relaxation noise component with a value for $f_c$ of $\sim$10–20 Hz (Van Driessche et al., 1991), referred to as a low-frequency Lorentzian. In this study we extended this finding and reported that spontaneous Lorentzian components with substantially higher $f_c$ values (60–120 Hz) also occur (Fig. 3 B and Table III). At this point it may be useful to digress on the relative occurrence of low- and high-frequency Lorentzians. Experiments for this study have been performed during a 2-yr period, using animals from different batches. We compiled the statistics on such a vast basis because different batches of animals sometimes conspicuously differ from one another with respect to the occurrence of low- or high-frequency Lorentzians. We determined the number of low- and high-frequency Lorentzians in skins subjected to Ca$^{2+}$-free mucosal Na$^+$-Ringer solutions containing amiloride. Out of a total of 205 experiments taken into consideration, 96 experiments yielded a PDS without an apparent Lorentzian feature, 58 experiments provided a low-frequency Lorentzian, 49 experiments produced a high-frequency Lorentzian, and 2 experiments allowed fits with a double Lorentzian. However, despite the fact that only very rarely was a double Lorentzian visible, almost every tissue (90%) did express both channel types, as can be inferred from its sensitivity to both UO$_2^{2+}$ and tetracaine. We found that PDSs containing a low-frequency Lorentzian were markedly influenced by UO$_2^{2+}$. Indeed, 100 μM of this divalent caused the disappearance of the original Lorentzian component together with a substantial reduction of background noise, so as to reveal another Lorentzian component with higher $f_c$ (Figs. 2 B and 6 B, and Table III). On the other hand, PDSs
displaying a high-frequency Lorentzian feature were not essentially affected, in that the divalent, at a concentration of 100 μM, only moderately suppressed the Lorentzian already present without changing $f_c$ (Figs. 3 B and 4 B). At this point, the important question that emerged was whether the Lorentzian with higher $f_o$ unmasked by UO$_2^{2+}$ (Fig. 2 B), found its origin in the same gating process as the high-frequency Lorentzian observed in the absence of UO$_2^{2+}$ (Figs. 3 B and 4 B) or, alternatively, reflected UO$_2^{2+}$-induced changes in the open-closed kinetics of type S channels. Solving this problem was hampered by the existence of large 1/f excess noise in the absence of mucosal Ca$^{2+}$. More precisely, under our standard experimental conditions it appeared impossible to estimate the extent to which the low-frequency Lorentzian was depressed by UO$_2^{2+}$, and whether or not the corner frequency shifted during this process. However, we could find a set of appropriate experimental conditions that resolved these difficulties. The result, illustrated in Fig. 5, unequivocally establishes that (a) 100 μM, and even 10 μM UO$_2^{2+}$ for that matter, inhibits the low-frequency Lorentzian by 75% at least; (b) the open-closed kinetics of the low-frequency Lorentzian component are not modified by UO$_2^{2+}$; (c) UO$_2^{2+}$ does not by itself induce additional fluctuation, because the low-frequency Lorentzian is suppressed without the appearance of a high-frequency Lorentzian. We conclude that the high-frequency Lorentzian unmasked by UO$_2^{2+}$ (Figs. 2 B and 6 B) must represent an independent gating process besides the low-frequency one, and cannot be attributed to UO$_2^{2+}$-induced changes in the low-frequency gating process. Also, together with the irreversibility of the depression of the low-frequency Lorentzian by UO$_2^{2+}$, the previous result strongly suggests that the mode of action of UO$_2^{2+}$ on type S channels consists of an irreversible binding resulting in complete occlusion of the channels. Thus, the decrease of $f_c$ with UO$_2^{2+}$ results from a reduction in the number of conductive units, and not from a shift in the channels’ open probability.

**Two Channel Hypothesis Versus Two Gating Modes of Only One Channel Protein**

So far, we argued that our data can be understood in terms of two gated channels with different kinetics. On the other hand, it is conceivable that the low- and high-frequency Lorentzians reflect different gating modes of one and the same channel type. In any case, a crucial element that has to be accounted for is that fluctuation in Cs$^+$ current through the cation-selective channels apparently gives rise to high-frequency Lorentzians only (Figs. 7 B and 8 B). On the other hand, the Ca$^{2+}$-blockable channels in toad urinary bladder give rise to relaxation noise in the high-frequency part of the PDS that is insensitive to UO$_2^{2+}$ (Fig. 9). Therefore, they seem to belong to the same class as type F channels in frog skin. Finally, it must be repeated that a different UO$_2^{2+}$ sensitivity between the two classes of Lorentzians shows up very clearly. 10 μM UO$_2^{2+}$ affects only the low-frequency Lorentzian, whereas at higher concentrations both Lorentzians are depressed. We want to stress, therefore, that UO$_2^{2+}$ cannot be viewed as an all-or-none agent, but that in a certain range of concentrations it allows us to distinguish between two gating processes. The observation that higher amounts of UO$_2^{2+}$ inhibit both gating processes might be interpreted in favor of one channel type with two gating modes. The data with Cs$^+$ and with toad urinary bladder, on the other hand, favor the view that two different
channel types are present in the frog skin. A high-frequency component apparently exists in its own right in toad urinary bladder as well as in frog skin (Fig. 4), suggesting that a separate entity is associated with this kind of noise. It is not straightforward to assume that one and the same channel would display two gating modes in frog skin and only one in toad urinary bladder. Also, the result with Cs+ is more easily understood in terms of two channel types, one permeable (type F), the other impermeable (type S) to Cs+, than in terms of two modes of operation of the channel, one permitting Cs+ to pass and the other not. In the following paper (Desmedt et al., 1993), further differences in the pharmacology of both components will be established. Recapitulating, although it cannot strictly be proved, we favor the view of two classes of Ca2+-blockable, cation-selective channels in the frog skin, distinguished by their spontaneous gating kinetics, their sensitivity to UO2+2, and their permeability for Cs+.

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