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# Noise Analysis of the $K^+$ Current through the Apical Membrane of *Necturus* Gallbladder

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**Summary.** Current noise power spectra of the voltage-clamped ($V=0$) *Necturus* gallbladder, exposed to NaCl-Ringer's on both sides contained a relaxation noise component, which overlapped with a $1/f^\alpha$ noise component, with $\alpha$ being about 2. Substitution of all $Na^+$ by $K^+$ on either the serosal or mucosal side increased the relaxation as well as the $1/f^\alpha$ noise component considerably. In *Necturus* gallbladder both noise components are reduced by addition of 10 mM 2,4,6-triaminopyrimidine (TAP) or 5 mM of tetraethylammonium ($TEA^+$) added to ification of the mucosal solution to pH 5 and lower. Five mM of tetraethylammonium ($TEA^+$) added to the mucosal solution, abolished $K^+$ relaxation noise and decreased the $1/f^\alpha$ noise component. Applying a $Cs^+$ concentration gradient across the epithelium did not yield relaxation noise. However, if $Rb^+$ was substituted for all $Na^+$ on one side, a Lorentzian noise component appeared in the spectrum. Its plateau was smaller than with KCl-Ringer's on the respective side. These data confirm the existence of fluctuating $K^+$ channels in the apical membrane of the *Necturus* gallbladder. Furthermore it can be concluded that these channels have the permeability sequence $K^+ > Rb^+ > Cs^+$. The inhibition of the fluctuations by mucosal acidification indicates the existence of acidic sites in the channel. The single-channel conductance was estimated to be between 6.5 and 40 pS.

**Key words:** gallbladder, noise analysis, potassium channel, apical membrane, *Necturus*

The existence of fluctuating $K^+$ channels in the apical membrane of the toad gallbladder was demonstrated with fluctuation analysis (Van Driessche & Gögelein, 1978). The power spectrum of the transepithelial fluctuations in current contained a Lorentzian component when the epithelium was exposed to NaCl-Ringer's on both sides (control conditions). This relaxation noise component was considerably enhanced when NaCl-Ringer's was substituted by KCl-Ringer's on either the serosal or mucosal side. It was concluded that the underlying relaxation process was related to a $K^+$ movement across $K^+$ permeable channels in the apical membrane, which open and close randomly. The relaxation noise component, observed under control conditions, was assumed to be due to a $K^+$ efflux into the mucosal bathing solution. This $K^+$ movement can be expected, as intracellular $K^+$ activity is higher than the value expected from passive distribution (Zeuthen, 1978; Reuss & Weinman, 1979), and as the apical membrane is predominantly $K^+$ permeable (Hénin & Cremaschi, 1975; Reuss & Finn, 1975*a*, *b*; Van Os & Slegers, 1975). The experiments described in this study were designed to investigate the effects of possible inhibitors on the fluctuating $K^+$ channels, such as 2,4,6-triaminopyrimidine ($TAP^+$), tetraethylammonium ($TEA^+$), $Ba^{2+}$ and $Cs^+$ ions, as well as the effect of protons. In addition, the permeability of the $K^+$ channels to $Rb^+$ and $Cs^+$ ions was investigated. The properties of the $K^+$ channels in the apical membrane of the gallbladder are compared with those of $K^+$ channels in excitable tissues. This allows us to propose a pore model for the apical $K^+$ channels in the gallbladder. The experiments were performed with the gallbladder of *Necturus maculosus*, as the properties of the cell membranes as well as the intracellular ion activities of the bladder of this species were studied by numerous investigators with different electrophysiological methods. Taking account of such data makes it possible to estimate the single-channel conductance and the pore density of the apical $K^+$ channels of this tissue.

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## Materials and Methods

*Necturi* were obtained from Mogul-Ed. Co, (Oshkosh, Wisconsin). They were kept at 15 °C in aerated water and were fed with live goldfish. The animals were anesthetized with 0.1% MS 222 (Sandoz, Basel). The gallbladder was excised, cut open and rinsed free of bile. Then the epithelium was stretched out moderately, glued on its serosal side onto a plexiglas ring and was mounted between two halves of a Lucite chamber. The epithelial surface exposed to the bathing solutions was 0.12 $cm^2$. In order to minimize edge damage, the epithelium was sealed with soft silicon rubber rings. Air, bubbled through the media, provided both oxygenation and stirring. In order to prevent mechanical movement of the bladder, the mucosal and serosal solutions were aerated separately while the other compartment was closed off. Bubbling was stopped and both compartements were locked during recording of the noise signals.

The solutions used under control conditions, further referred to as NaCl-Ringer's, had the following composition (in mM): 109 NaCl, 2.5 KCl, 0.9 $CaCl_2$, 2.4 $NaHCO_3$ (pH = 8.4, after bubbling with air). KCl-, RbCl-, and CsCl-Ringer's solutions were made by replacing all $Na^+$ by the respective ion.

2,4,6-triaminopyrimidine (TAP) was purchased from Aldrich Europe (Beerse, Belgium). In experiments containing $TAP^+$ the pH of the solution was adjusted to 6 by addition of HCl. TAP was added in amounts of 10 mM (8.5 mM $TAP^+$) to both bathing solutions. To investigate effects of pH variations, the following buffers were used (Wright & Diamond, 1968): pH 9.4: 1.6 mM glycin and NaOH; pH 7.4 and 6.0: 2.5 mM $K_2HPO_4 - KH_2PO_4$; pH 5.0 – 3.4: 1.6 mM potassium phtalate and NaOH or HCl. Experiments with $Ba^{2+}$ ions and the respective control measurements were performed in $Ca^{2+}$-free Ringer's solution where bicarbonate was replaced by 5 mM Tris buffer. All experiments were performed at room temperature (about 22 °C).

The presented biionic and dilution potentials were corrected for liquid junction potentials, which were calculated by the method described by Barry and Diamond (1970). Potentials are referred to the serosal side. Mean values are given with standard errors of the mean. Statistical comparisons concerning the significance of differences between mean values were made with the paired *t*-test.

### *Electrical Set-up and Methods of Fluctuation Analysis*

Details about the recording and analysis of noise signals are described elsewhere (Van Driessche & Lindemann, 1978; Van Driessche & Zeiske, 1980*a*). They will be briefly mentioned here. In order to avoid aliasing the amplified signal was filtered with a 48 dB/octave low-pass filter (Rockland 852) with its cut-off frequency adjusted to 220 Hz. To remove signals below the smallest analyzed frequency the signal was also filtered with a 48 dB/octave high-pass filter with a cut-off frequency of 0.07 Hz. This cut-off frequency is sufficiently below the fundamental frequency (0.25 Hz) in order to avoid its attenuation. The filtered signal was digitized at intervals of 2 msec, temporarily stored in a digital semiconductor memory and finally stored on a digital magnetic tape. One record consisted of 2048 data points (4096 msec length). The power spectra were computed on a PDP 11/34 computer with a Fast Fourier Transform program (Decus 179). One spectrum represents the mean of 40 records. The spectral densities were plotted as a function of the frequency on a double logarithmic scale on the monitor of a graphic display processor (VT11, Digital Equipment). As the power density of the fluctuation in current arising from the open-close kinetics of ion-selective channels is proportional to the area of the tissue (Stevens, 1972; Chen & Hill, 1973), the power density was divided by this area. The curves in the power spectra were fitted by a method of nonlinear least-squares (Van Driessche & Zeiske, 1980*b*.) The experimental data could be fitted either with a sum of one Lorentzian and an $A/f^\alpha$ component or with a sum of two Lorentzians and an $A/f^\alpha$ component. The $A/f^\alpha$ component was included in the fitting procedure in order to describe the intense low-frequency (LF) noise component which overlapped with the relaxation noise under most experimental conditions. Possible origins of this LF noise will be addressed in the Discussion section. Power spectra were fitted by a Lorentzian when the relaxation noise component was only slightly disturbed by LF noise. The data points departing from the Lorentzian in the LF region could be excluded from the fitting procedure. It will be shown in this study that spectral values above 10 Hz deviated in most cases from a Lorentzian curve. Therefore the possibility was provided to exclude these points from the fitting procedure. The points to be fitted were demarcated by two markers, which could be moved along the frequency axis. Power spectra and fitted curves demonstrated in this study were plotted with a Hewlett Packard plotter (7221A).

Current and voltage-sensing electrodes consisted of Ringer's-filled agar bridges which were connected via a compartment filled with Ringer's solution, to sintered Ag/AgCl electrodes (Annex Research, Santa Ana, Calif.). Current and voltage electrodes had a distance from the epithelium of 30 and 5 mm, respectively. The electrical resistance of each current electrode was about 1 kΩ and that of each voltage electrode was about 2.5 kΩ. The resistance of the Ringer's solution between the voltage electrodes ($R_c$) was about 80 Ωcm$^2$.

The methods used for the impedance measurements are described in the following paper (Gögelein & Van Driessche, 1981).

## Results

### *Fluctuation Analysis with NaCl-Ringer's on Both Sides*

In *Necturus* gallbladder exposed to NaCl-Ringer's on both sides, the spectral values in current and voltage noise spectra exceeded those of the amplifier background noise considerably in the low and middle frequency range. A typical current fluctuation pattern, recorded at zero clamp voltage, is shown in Fig. 1*a*. In the power spectrum an $A/f^\alpha$ component dominated at low frequencies, but frequently a relaxation noise component was indicated (Fig. 2). In 10 of 48 bladders the latter noise type was pronounced and could be fitted with Lorentzian curves. An example of such fitted power spectra is demonstrated in Fig. 2. It shows spectra of current noise $S_I(f)$ and voltage noise $S_V(f)$, as well as the magnitude of the impedance as a function of the frequency. The current noise spectrum was recorded by clamping the epithelium at zero potential. Clamping at the small transepithelial potential yielded identical results. The voltage noise spectrum and the impedance were recorded under open-circuit conditions. In addition, Fig. 2 demonstrates a current noise spectrum, recorded after replacing the preparation by a dummy network (*see* inset of Fig. 2). The electrical parame-

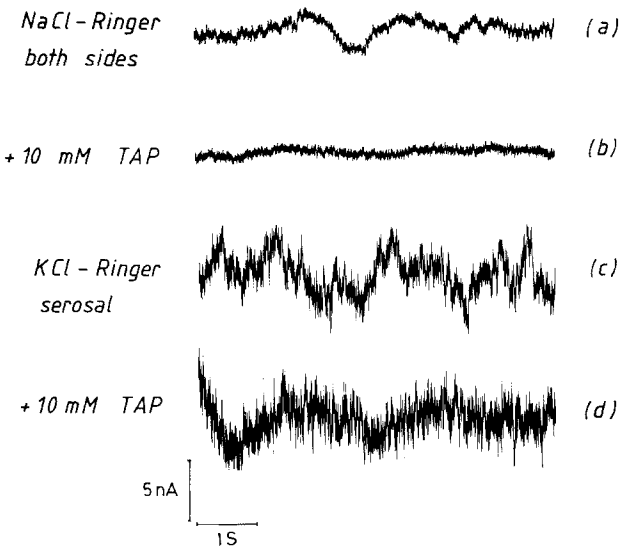


**Fig. 1.** Current fluctuation patterns of voltage-clamped *Necturus* gallbladders. In (*a*) and (*b*) the epithelium was clamped at $V = 0$ mV, in (*c*) and (*d*) it was clamped at the biionic potential (not corrected for liquid junction potential)

ters of this network represent the passive electrical equivalent characteristics of the epithelium and the measuring electrodes. In the LF region ($f < 10$ Hz) the data points of the power spectra, recorded with the epithelium, could be fitted by the sum of a Lorentzian and an $A/f^\alpha$ component. If spectral values at frequencies greater than about 10 Hz were included in the data block to be fitted, systematic deviations between the fitted curve and the data points were observed. Therefore data points above about 10 Hz were excluded from the fit. The results of 10 experiments are summarized in Table 1 (row 1). The deviation of the data points from the fitted curve above 10 Hz could be due either to a frequency dependent attenuation effect or to the existence of another relaxation noise component. Fits by two superimposed Lorentzians and an $A/f^\alpha$ component described the data points in the entire frequency range adequately, excluding the spectral values in the region where amplifier noise becomes important (not shown).

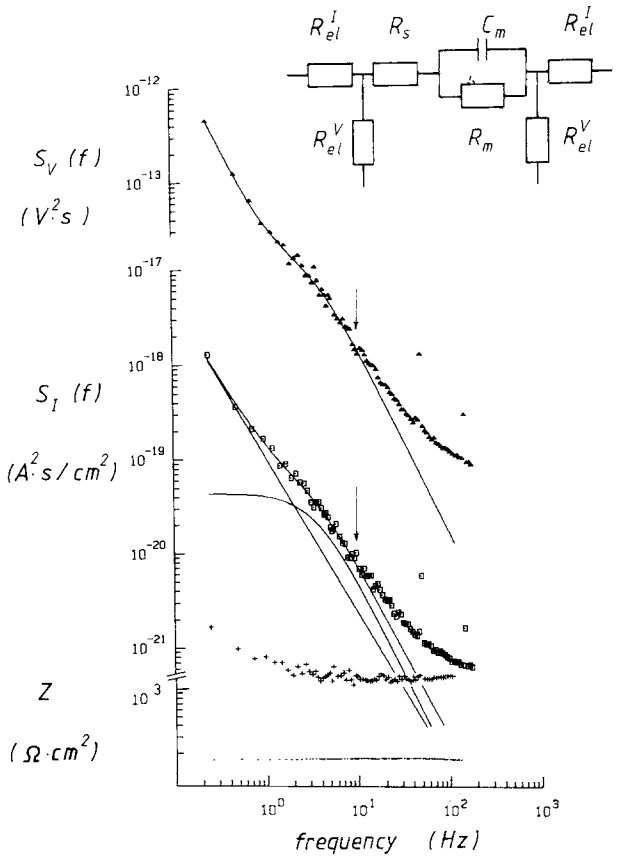


**Fig. 2.** Power spectra of voltage $S_V(f)$ (▲) and current $S_I(f)$ (■) fluctuations and the magnitude of the impedance $Z$ recorded under control conditions. The fits consist of the sum of a Lorentzian and an $A/f^\alpha$ component. Data points on the right of the arrows were excluded from the fitting procedure. In the current noise spectrum the components of the fitted curve are shown separately. In addition, the spectrum of the current noise produced by the amplifier system (+) is demonstrated. It was recorded by replacing the preparation by a dummy network, as depicted on the inset of the Figure. The parameters are: $R_{el}^I = 1$ kΩ, $R_{el}^V = 2.5$ kΩ, $R_s = 750$ Ω, $R_m = 600$ Ω, and $C_m = 1$ μF

The impedance plot in Fig. 2 shows that $|Z|$ is nearly constant in the analyzed frequency range. Wanke, DeFelice and Conti (1974) demonstrated that, within experimental accuracy, in squid axon membrane the relation

$$S_V(f) = |Z(f)|^2 * S_I(f) \quad (1)$$

**Table 1.** Plateau values $S_o$, corner frequencies $f_c$ and values of the noise component $A/f^\alpha$ of *Necturus* gallbladder under various conditions. ($N$ = number of observations)

| Conditions | $S_o$ ($A^2 \cdot sec/cm^2$) | $f_c$ (Hz) | $A$ ($A^2/cm^2$) | $\alpha$ | $N$ |
|---|---|---|---|---|---|
| NaCl-Ringer's both sides | $(7.8 \pm 2.7) \times 10^{-20}$ | $3.2 \pm 0.3$ | $(1.2 \pm 0.2) \times 10^{-19}$ | $2.1 \pm 0.1$ | 10 |
| NaCl-Ringer's +10 mM TAP both sides | $(4.8 \pm 1.3) \times 10^{-20}$ | $6.3 \pm 0.7$ | $(2.1 \pm 1.1) \times 10^{-20}$ | $2.2 \pm 0.3$ | 8 |
| KCl-Ringer's serosal | $(3.4 \pm 0.6) \times 10^{-18}$ | $2.1 \pm 0.3$ | $(1.5 \pm 0.3) \times 10^{-18}$ | $2.4 \pm 0.2$ | 9 |
| KCl-Ringer's serosal +10 mM TAP both sides | $(1.0 \pm 0.3) \times 10^{-18}$ | $5.1 \pm 0.5$ | | | 18 |
| KCl-Ringer's mucosal +10 mM TAP both sides | $(6.9 \pm 1.7) \times 10^{-19}$ | $4.3 \pm 0.8$ | $(2.4 \pm 0.7) \times 10^{-19}$ | $1.9 \pm 1.2$ | 7 |

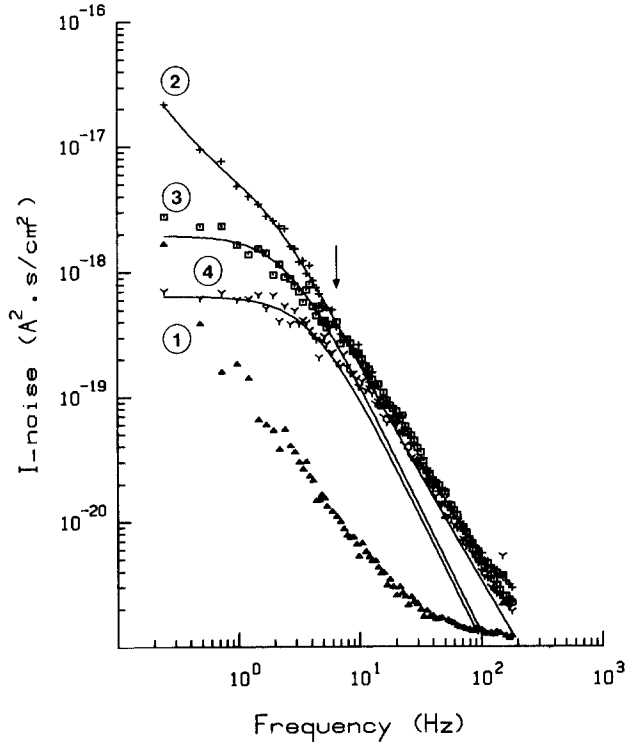


**Fig. 3.** Power spectra of the current fluctuations recorded under control conditions (spectrum 1, ▲) and with KCl-Ringer's on the serosal side (spectrum 2, +). Spectra (3) and (4) were recorded 25 min (■) and 60 min (Y), respectively, after addition of 10 mM TAP to both sides, when KCl-Ringer's was present on the serosal side

can be applied. It was demonstrated (Van Driessche & Gögelein, 1980) that this relation can be used for transepithelial noise measurements, even when the paracellular shunt resistance is much smaller than the resistance of the cell membranes. Eq. (1) predicts that current and voltage noise spectra have the same shape, if $|Z(f)|$ is frequency independent in the respective frequency range. This is confirmed by the power spectra shown in Fig. 2. The quantitative validity of Eq. (1) was tested at the arbitrarily chosen frequency point of 10 Hz. No significant difference between the mean values of $S_V/S_I$ and $|Z|^2$ was found (8 observations). Hence, it can be concluded that Eq. (1) can be applied to fluctuations in *Necturus* gallbladder exposed to NaCl-Ringer's on both sides.

### *Effects of a Transepithelial $K^+$ Concentration Gradient*

Substitution of all $Na^+$ by $K^+$ on either the serosal or mucosal side increased the amplitudes of the relaxation as well as LF noise components appreciably. Fig. 1*c* demonstrates a fluctuation pattern and Fig. 3 (curve 2) a power spectrum, recorded with serosal KCl-Ringer's. Spectrum 1 in Fig. 3 was recorded under control conditions. As an $A/f^\alpha$ noise component dominated at low frequencies in the demonstrated example, the data points were not fitted. With serosal KCl-Ringer's this LF noise increased considerably, and in addition the Lorentzian component appeared more clearly (curve 2). At frequencies below 10 Hz the data points could be well fitted by the sum of a Lorentzian and an $A/f^\alpha$ component, whereas the data points deviate significantly from the fitted curve at higher frequencies. Because of the increased LF noise, fits with the sum of a Lorentzian curve and an $A/f^\alpha$ component were possible in only 9 of 16 bladders. The results of these fitted experiments are summarized in Table 1 (row 3). The plateau values increased considerably after KCl-Ringer's was substituted for NaCl-Ringer's on the serosal side, whereas the corner frequencies are not significantly different. The enhanced intensity of the LF noise component is described by the increase of the $A$ value. When all $Na^+$ in the mucosal solution was replaced by $K^+$, the intensity of the fluctuations increased as with serosal KCl-Ringer's. Because of intense LF noise it was not possible to make Lorentzian fits of spectra recorded with mucosal KCl-Ringer's.

Replacing $Na^+$ by $K^+$ gradually on either the serosal or mucosal side yielded results similar to those described in toad gallbladder (Van Driessche & Gögelein, 1978). In order to suppress LF noise and to enable fits with Lorentzian curves of these spectra, 10 mM TAP was present on both sides (*see* following section). When $Na^+$ was replaced gradually by $K^+$ on the serosal side, the plateau of the Lorentzian component increased. However, raising mucosal $K^+$ to 36 mM abolished the relaxation noise component observed with NaCl-Ringer's on both sides. The Lorentzian reappeared at a mucosal $K^+$ concentration of 57 mM and increased with further increasing mucosal $K^+$ concentration. We concluded that increasing serosal $K^+$ augments the $K^+$ efflux across the apical membrane. On the other hand, an increase of mucosal $K^+$ concentration to 36 mM is assumed to abolish the electrochemical $K^+$ gradient, existing across the apical membrane under control conditions (Zeuthen, 1978; Reuss & Weinman, 1979). A further increase of mucosal $K^+$ causes a $K^+$ influx across the fluctuating channels in the apical membrane, and could account for the reappearance of the Lorentzian curve, at high mucosal $K^+$ concentration.

### *Effects of $TAP^+$*

10 mM TAP was added to the bathing solutions of gallbladders which yielded a Lorentzian noise com-

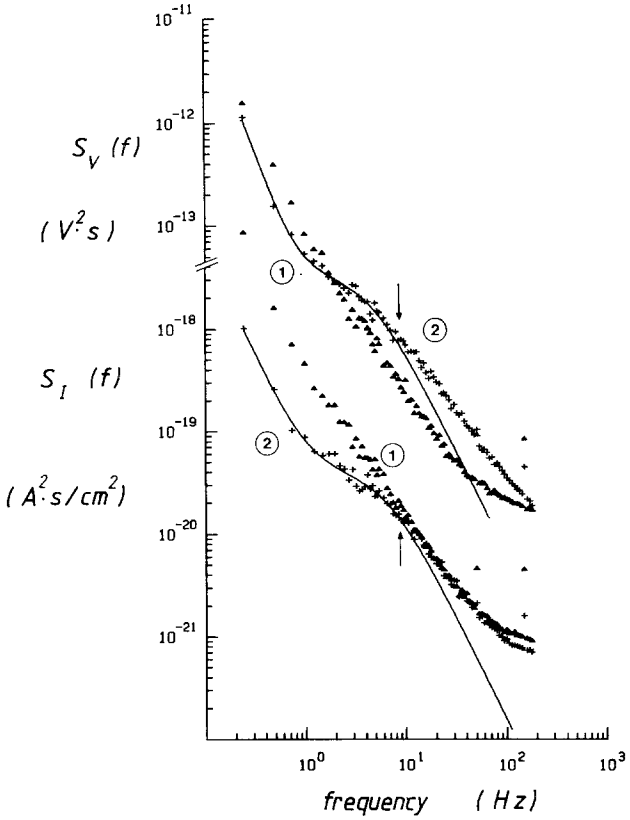


**Fig. 4.** Power spectra of voltage and current fluctuations recorded under control conditions (spectra 1, ▲) and 15 min after addition of 10 mM TAP to both sides (spectra 2, +)

ponent in the spectrum of the current fluctuations under control conditions. The LF noise component and the plateau value $S_o$ then decreased significantly whereas the corner frequency $f_c$ increased (Table 1, row 2). As $TAP^+$ decreased the LF noise component, some spectra could be fitted by the sum of a Lorentzian and an $A/f^\alpha$ component only after addition of $TAP^+$. This decrease in LF noise is also obvious in the fluctuation pattern demonstrated in Fig. 1*b*, as well as in the decrease of the parameter $A$. However, the slope $\alpha$ did not change significantly (Table 1, row 2). Fig. 4 shows, as an example, power spectra of voltage and current noise before and after addition of $TAP^+$, when the bladder was bathed with NaCl-Ringer's on both sides. In this example, the data points were fitted by the sum of a Lorentzian and an $A/f^\alpha$ component only after addition of $TAP^+$ (curves 2). In the voltage noise spectrum the relaxation noise component became evident only after application of $TAP^+$. In the current noise power spectrum the data points decreased significantly below 10 Hz, whereas no significant change was observed at higher frequencies.

The influence of $TAP^+$ was also investigated when $K^+$ relaxation noise was enhanced by KCl-Ringer's on either the serosal or mucosal side. With KCl-Ringer's on either side, the addition of $TAP^+$ to both sides reduced the LF noise component significantly. Therefore fits with a Lorentzian and an $A/f^\alpha$ component were in many cases only possible after application of $TAP^+$. Particularly, the spectra recorded with mucosal KCl-Ringer's could be fitted only after addition of $TAP^+$. In 50% of all cases, when KCl-Ringer's was present on the serosal side, the decrease of the LF noise was so pronounced that the spectra could be fitted solely by a Lorentzian. The $S_o$ and $f_c$ values, summarized in Table 1 (row 4) demonstrate a decrease in $S_o$ and an increase in $f_c$ values after addition of $TAP^+$, as observed with NaCl-Ringer's on both sides. It was often observed that the spectral values above 10 Hz increased slightly after application of $TAP^+$. Therefore the deviation of the data points above 10 Hz from the fitted curve became more pronounced. This increase in high-frequency fluctuations with $TAP^+$ is also visible in the fluctuation pattern of Fig. 1*d*. Table 1 shows that, in the presence of $TAP^+$, the $S_o$ values with mucosal KCl-Ringer's are smaller than with serosal KCl-Ringer's whereas the $f_c$ values are comparable. In the same way as with serosal KCl-Ringer's, the spectral values deviated significantly from the fitted curve at frequencies above 10 Hz (not shown). The effects of $TAP^+$ were reversible.

$TAP^+$ had a time-dependent effect on current fluctuations. We investigated this effect in detail when KCl-Ringer's was present on the serosal side. As demonstrated in Fig. 3, the plateau value decreased with time, whereas the corner frequency increased. In order to investigate these effects statistically, we compared $S_o$ and $f_c$ values recorded about 20 min and about 80 min after application of $TAP^+$ (6 observations). $S_o$ decreased significantly from $(1.1 \pm 0.3) \times 10^{-18}$ $A^2$ sec/$cm^2$ to $(3.8 \pm 0.9) \times 10^{-19}$ $A^2$ sec/$cm^2$ ($p < 0.01$), whereas $f_c$ increased from $(4.1 \pm 0.7)$ Hz to $(8.6 \pm 1.1)$ Hz ($p < 0.005$). The latter observation shows that the open-close kinetics of the $K^+$ channels are influenced by application of $TAP^+$.

### *Permeability of the Fluctuating Channels to $Rb^+$ and $Cs^+$ Ions*

In order to estimate the permeability of the apical $K^+$ channels in *Necturus* gallbladder epithelium for $Cs^+$ and $Rb^+$ ions, all $Na^+$ on either the serosal or mucosal side was replaced by the respective ions. In order to reduce LF noise and to enable Lorentzian fits, $TAP^+$ was present on both sides in all experiments reported in this section. Experiments with RbCl- and CsCl-Ringer's were preceded and fol-

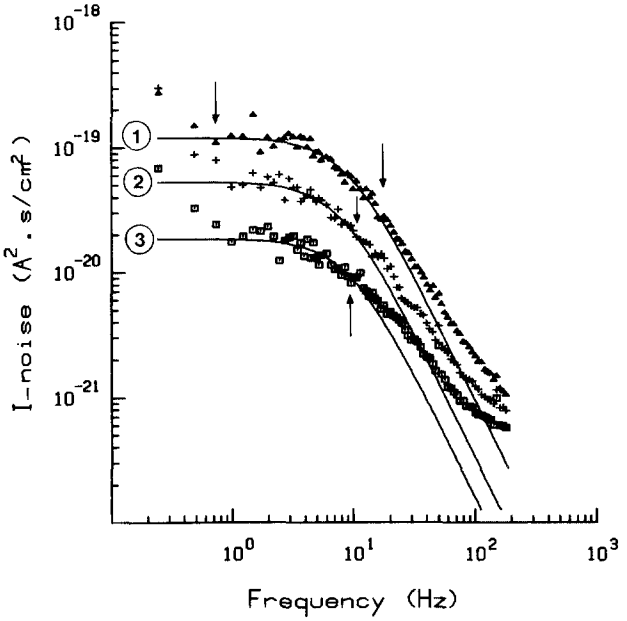


**Fig. 5.** Power spectra of current noise with KCl-Ringer's (▲) or RbCl-Ringer's (+) on the serosal side (spectra 1 and 2, respectively) and with NaCl-Ringer's on both sides (spectrum 3, ■). In all cases, 10 mM TAP was present on both sides. Only data points between the arrows were used to fit the spectra by Lorentzian curves

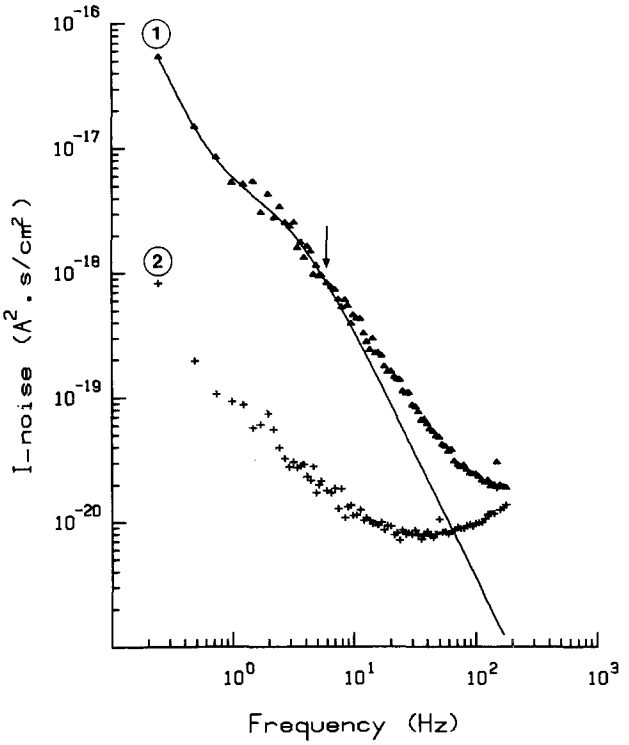


**Fig. 6.** Effect of 5 mM $TEA^+$ on the mucosal side on current noise spectra. Bathing solutions: NaCl-Ringer's on the mucosal and KCl-Ringer's on the serosal side (+TAP). Curve 1 (▲): no $TEA^+$; curve 2 (+): +$TEA^+$

lowed by periods with KCl-Ringer's on the side concerned. The measurements performed during this period were done to ascertain that the time dependent effects of $TAP^+$, arising during a series of experiments, could be neglected. When KCl-Ringer's was replaced by CsCl-Ringer's on either the serosal or mucosal side no relaxation noise could be detected (2 observations of each kind). As discussed in the next section, small amounts (10 mM) of $Cs^+$ added to the mucosal solution did not exert an inhibitory effect on the $K^+$ current fluctuations. Therefore, it is unlikely that the absence of a Lorentzian component would be due to a blocking effect of $Cs^+$ on a still existing $K^+$ flux through the apical membrane. On the other hand, it can be assumed that no essential $Cs^+$ movement occurs through the fluctuating $K^+$ channels.

When RbCl-Ringer's was present on either side of the epithelium, relaxation noise could still be observed. A representative current noise spectrum is shown in Fig. 5. The plateau value with serosal RbCl-Ringer's was significantly smaller than with serosal KCl-Ringer's (curves 2 and 1, respectively) but it was still larger than with NaCl-Ringer's on both sides (curve 3). Replacement of mucosal KCl-Ringer's by RbCl-Ringer's yielded similar results. The corner frequencies, as well as the transepithelial resistances, obtained with KCl- and RbCl-Ringer's were not significantly different (5 observations). Therefore it is unlikely that the Lorentzians observed with RbCl-Ringer's are caused by a blocking effect of $Rb^+$ of a possible $K^+$ efflux through the apical $K^+$ channels. Consequently, these data indicate that the $Rb^+$-dependent Lorentzians are due to a $Rb^+$ flux through the apical $K^+$ channels which seem to be less permeable to $Rb^+$ than to $K^+$ ions and that the open-close kinetics of the $K^+$ channels are not influenced by $Rb^+$ ions.

### *Effects of $TEA^+$*

As was observed in toad gallbladder (Van Driessche & Gögelein, 1978), $TEA^+$ proved to be a potent blocker of $K^+$ relaxation noise in *Necturus* gallbladder. A typical experiment is demonstrated in Fig. 6, where 5 mM $TEA^+$ was added to the mucosal bathing solution, after $K^+$ fluctuations were enhanced by substitution of $Na^+$ by $K^+$ on the serosal side. The spectrum with $TEA^+$ (curve 2) was recorded 5 min after its application. With $TEA^+$ the relaxation noise component was depressed to such an extent that the spectrum could not be fitted by Lorentzian

**Table 2.** Effect of 5 mM $TEA^+$, added to the mucosal or serosal side, on the Na-K biionic potential $V_{Na/K}$ and transepithelial resistance $R_t$ of *Necturus* gallbladder. (Number of observations are given in brackets)

| Conditions | $V_{Na/K}$ (mV) | $R_t$ ($\Omega cm^2$) |
|---|---|---|
| KCl-Ringer's serosal (4) | 18.7±2.8 | 102±16 |
| +5 mM $TEA^+$ mucosal (4) | 14.0±1.8 | 127±20 |
| Difference | −4.7±1.3 | 21± 6 |
| $p$ | <0.0125 | <0.0025 |
| KCl-Ringer's serosal (4) | 14.5±1.3 | 128±2 |
| +5 mM $TEA^+$ serosal (4) | 13.9±1.1 | 124±2 |
| Difference | 0.5±0.3 | −4±2 |
| $p$ | ns | ns |

curves. It should be pointed out that $TEA^+$ depressed also the noise component above 10 Hz as well as the LF noise. Similarly a Lorentzian noise component, present with NaCl-Ringer's on both sides, was abolished by mucosal $TEA^+$ (not shown). In gallbladders exposed to KCl-Ringer's on the mucosal and NaCl-Ringer's on the serosal side, the Lorentzian noise component, recorded in the presence of $TAP^+$, was abolished after addition of 5 mM $TEA^+$ to the mucosal solution. Added to the serosal solution, $TEA^+$ had no effect on the current fluctuations. 5 mM $TEA^+$ on the mucosal side also abolished $Rb^+$-dependent relaxation noise. The inhibitory effect of mucosal $TEA^+$ was completely reversible. Unlike in squid axon membrane (Moore, Fishman & Poussart, 1979) no $TEA^+$-induced relaxation component at high frequencies was observed in the gallbladder. However, such an effect could be masked by amplifier noise, which becomes dominant at frequencies greater than about 100 Hz in our preparation.

Besides the effect on fluctuating $K^+$ channels mucosal $TEA^+$ increased the transepithelial resistance $R_t$ and decreased the Na-K-biionic potential $V_{Na/K}$. Mean values of these parameters, recorded before and after application of $TEA^+$ to the mucosal side, are presented in Table 2 (rows 1 and 2). The 2:1 NaCl-dilution potential (50% of mucosal $Na^+$ was replaced isoosmotically by sucrose) was (11.4 ±1.0) mV in absence of $TEA^+$ and (11.3±1.0) mV in presence of 5 mM $TEA^+$ on the mucosal side (5 observations). Thus, in contrast to the effect of $TAP^+$ (Moreno, 1975), the NaCl-dilution potential did not change significantly with $TEA^+$ ($p>0.7$). When added to the serosal side $TEA^+$ affected neither $R_t$ nor the biionic potential significantly (Table 2). It was demonstrated that the transepithelial conductance is mainly due to the conductance of the paracellular pathway (Frömter, 1972) and that this shunt pathway has cationic selectivity (Reuss & Finn, 1975*a*; Van Os & Slegers, 1975). Consequently the increase in $R_t$ with mucosal $TEA^+$ indicates that the agent also decreases the shunt cationic permeability.

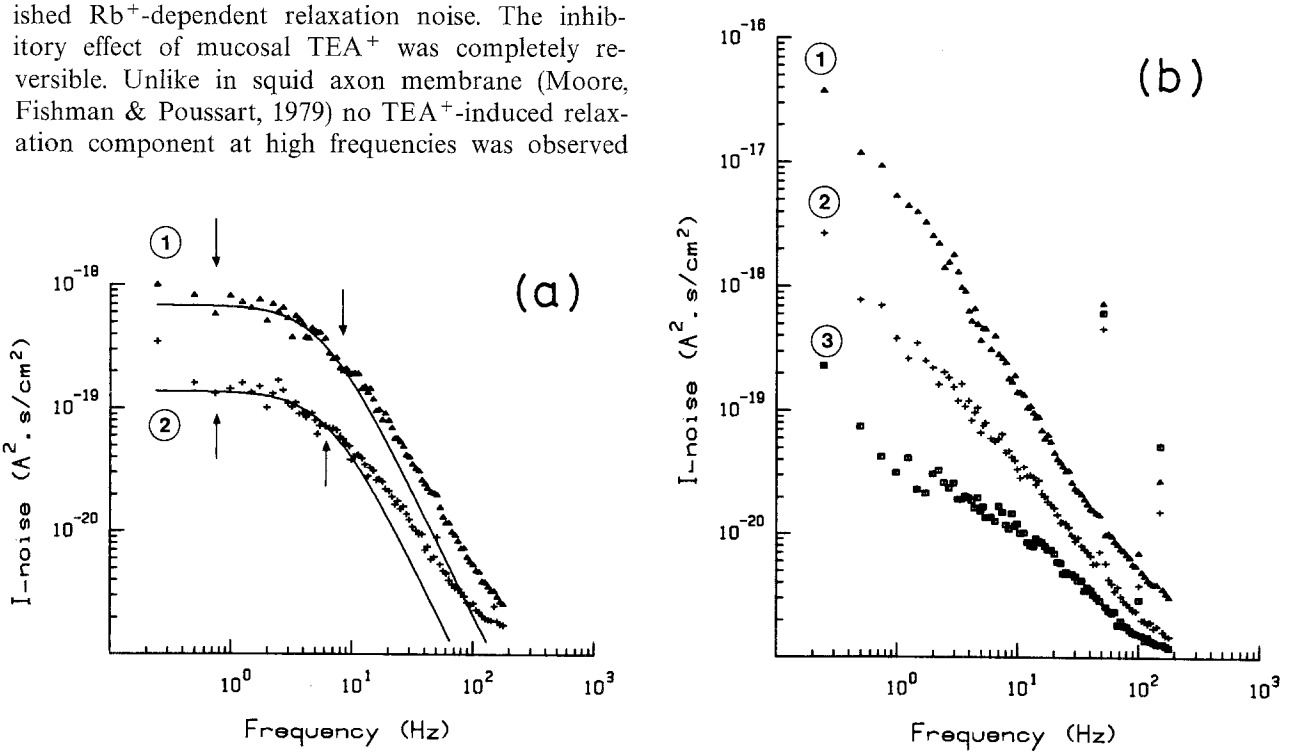


**Fig. 7.** Effect of mucosal $Ba^{2+}$ (*a*) and of acidification of the solutions on both sides (*b*) on current noise spectra. In (*a*) spectrum 1 (▲) was recorded with serosal KCl-Ringer's and spectrum 2 (+) after addition of 5 mM $Ba^{2+}$ to the mucosal solution (+TAP in both cases). In (*b*) the epithelium was exposed to KCl-Ringer's on the serosal side. Spectrum 1 (▲) was recorded at pH 7.4, spectra 2 (+) and 3 (■) at pH 5.0 and 4.4, respectively

*Effects of Inorganic Cations and pH*

Van Driessche and Zeiske (1980*a*) reported that in frog skin epithelium 10 mM $Cs^+$ blocked $K^+$ relaxation noise completely. However, the addition of 10 mM $Cs^+$ to the mucosal side had no effect on $K^+$ current noise in *Necturus* gallbladder. Similarly, the administration of 5 mM $CaCl_2$ to the mucosal side did not affect the noise features. However, when 5 mM $Ba^{2+}$ was added to the mucosal solution of *Necturus* gallbladders bathed with KCl-Ringer's on the serosal side, the plateau value decreased whereas the corner frequency did not change significantly (4 observations). A typical example is shown in Fig. 7*a*. The effect of $Ba^{2+}$ was completely reversible. In contrast to observations in frog skin epithelium, performed by Van Driessche and Zeiske (1980*b*), we did not find a $Ba^{2+}$-induced relaxation noise component in *Necturus* gallbladder.

Noise measurements at different pH-values were performed with KCl-Ringer's on the serosal side. In most cases the pH was changed on both sides of the epithelium. Changing the pH only on the mucosal side yielded qualitatively similar results. A typical experiment is demonstrated in Fig. 7*b*. As no $TAP^+$ was present the power spectrum at pH 7.4 (curve 1) showed intense LF noise. Lowering the pH on both sides to 5 and 4.4 (curves 2 and 3) decreased the fluctuations markedly. The power spectra recorded at pH 7.4 and 6.0 were not significantly different (not shown). The decrease of the spectral values at was reversible pH = 5. At pH 4.4 reversibility was not complete in most cases, and at pH 3.4 the epithelium deteriorated frequently. It can be concluded that the fluctuating $K^+$ pores are not affected by protons in the pH range between 6 and 9.4 and that at pH 5 and lower the fluctuating $K^+$ current is reduce.

## Discussion

In this study we demonstrated that in *Necturus* gallbladder epithelium spontaneous $K^+$ relaxation fluctuations can be observed when the epithelium is exposed to NaCl-Ringer's on both sides. The intensity of the spectral values increased considerably when all $Na^+$ of either the serosal or mucosal side is replaced by $K^+$. $TEA^+$ showed to be a potent blocker of $K^+$ relaxation noise when applied to the mucosal side. As was the case for the toad gallbladder (Van Driessche & Gögelein, 1978) it can be concluded from these findings that the apical membrane of *Necturus* gallbladder contains fluctuating $K^+$-selective channels. In this paper we showed that the apical $K^+$ channels in *Necturus* gallbladder are partially blocked by $TAP^+$ (10 mM) and $Ba^{2+}$ (5 mM) as well as by increasing the mucosal proton concentration. Furthermore we showed that $Rb^+$ is less permeable than $K^+$ whereas $Cs^+$ ions are impermeable.

Experiments with rabbit gallbladder showed that $K^+$ relaxation noise could also be recorded in this tissue. $TEA^+$ added to the mucosal solution depressed the relaxation noise. In contrast to *Necturus* and toad gallbladder the corner frequency in rabbit gallbladder was about 34 Hz at 37 °C and about 11 Hz at 22 °C *(unpublished results)*. Hence, the relaxation time of the open-close reaction of apical $K^+$ channels in rabbit gallbladder is shorter than in cold-blooded animals.

*Attenuation of the Noise Signals*

Transepithelially recorded noise data give only indirect information about conductance fluctuations arising in cell membranes. A direct measurement of apical conductance fluctuations would require an ideal voltage clamp across this membrane. In this case the amplitudes of current and conductance fluctuations are directly proportional (equation 5.8 in Verveen & DeFelice, 1974), and their spectra have the same shape. The factor relating current and conductance noise amplitudes recorded under ideal clamp conditions, depends only on the driving force of the $K^+$ flux. Current fluctuations, expected from an ideal voltage clamp across a single cell membrane, will be designated henceforth as "intrinsic fluctuations". However, until now fluctuation analysis of a voltage-clamped single cell membrane in epithelia has not been performed. The relation between transepithelially recorded fluctuations and the intrinsic ones is complicated by the presence of the electrical equivalent parameters in series and in parallel with the noise source, such as the membrane capacitances, membrane resistances and electromotive forces (Van Driessche & Gögelein, 1980). Transepithelially recorded current and voltage fluctuations will be attentuated by these electrical equivalent parameters. Moreover, this attenuation may be frequency dependent, due to the existence of capacitive and inductive elements. Consequently, both the amplitude and shape of transepithelially recorded spectra may deviate from the intrinsic ones. Therefore we have to consider the possibility that apparent Lorentzians in the power spectra are artefacts, being due to such frequency dependent attenuation effects. For example, $A/f^\alpha$ diffusion noise could be depressed in the low-frequency part, so that an apparent Lorentzian curve becomes indicated. However, model calculations showed that no fre-

quency dependent attenuation effect can be expected when the epithelium is bathed with NaCl-Ringer's on both sides (Van Driessche & Gögelein, 1980). Furthermore, it is very unlikely that power spectra with a pronounced plateau value and no appreciable LF noise component, as shown in Figs. 3 and 5, arise because of attenuation effects.

The frequency independent attenuation of the intrinsic fluctuations is obtained by equation (21) in Van Driessche and Gögelein (1980), by setting $\omega=0$. If, in addition, the unspecific apical conductance $G_a^p$ as well as the electromotive force at the basolateral membrane $E_b$ are assumed to be zero, we obtain:

$$A=\left(\frac{(R_a+R_b)\cdot R_p+(R_a+R_b+R_p)\cdot R_s}{R_a\cdot R_p}\right)^2 \tag{2}$$

where $R_a$ and $R_b$ represent the apical and basolateral membrane resistance, respectively, $R_p$ the resistance of the paracellular pathway and $R_s$ the resistance in series with the epithelium. With the same assumptions mentioned above, the expressions for transepithelial current fluctuations $\Delta I_t$ and voltage fluctuations $\Delta V_t$ yield: (equations 15 and 20, in Van Driessche & Gögelein, 1980):

$$\Delta I_t=E_a\cdot\frac{R_a^2\cdot(R_p+R_s)\cdot R_p}{((R_a+R_b)\cdot R_p+(R_a+R_b+R_p)\cdot R_s)^2}\cdot\Delta G_a \tag{3}$$

and

$$\Delta V_t=E_a\cdot\frac{R_p\cdot R_a^2}{(R_a+R_b+R_p)^2}\cdot\Delta G_a \tag{4}$$

where $E_a$ is the apical membrane emf and $G_a$ the conductance of the apical membrane.

The attenuation factor of spectra recorded with NaCl-Ringer's on both sides can be estimated from $R_a$ and $R_b$ values obtained from the literature. Reuss and Finn (1975*a*) reported that apical and basolateral membrane resistance are 3350 and 2750 $\Omega cm^2$, respectively. As the permeability ratio of the apical membrane $P_K/P_{Cl}=2.9$ (Reuss & Finn, 1975*b*), the $K^+$-specific conductance of the apical membrane can be calculated: $R_a^K=4505\,\Omega cm^2$. The contribution of the apical $Na^+$ permeability in the estimation of $R_a$ can be neglected because $P_K/P_{Na}$ is about 45 (Reuss & Finn, 1975*b*). As the basolateral membrane is mainly $K^+$ permeable, we assumed $R_b$ to be equal to total basolateral membrane resistance determined by Reuss and Finn (1975*a*). The transepithelial conductance is approximately equal to that of the paracellular pathway, so that $R_p=R_t=80\,\Omega cm^2$. From our measurements we obtained $R_s=90\,\Omega cm^2$. Using these values we calculated $A=11.8$. This means that the spectral values recorded under normal conditions have to be multiplied by this factor in order to obtain the intrinsic ones. Recently, Frömter, Suzuki, Kottra and Kampmann (1981) reported preliminary values of 1220 and 201 $\Omega cm^2$ for the apical and basolateral membrane resistance, respectively. From these values we calculated $R_a=1640$ (assuming $P_K/P_{Cl}=2.9$) and $A=5.2$. The attenuation factor will be used below to calculate the single-channel conductance and pore density.

### *Do Fluctuating $K^+$ Channels Exist only in the Apical Membrane?*

In the gallbladder epithelium both the apical and basolateral membranes as well as the paracellular shunt pathway are predominantly $K^+$ permeable (Reuss & Finn, 1975*a*; Van Os & Slegers, 1975). Therefore it is conceivable that the relaxation noise, observed under normal conditions, is due to ionic $K^+$ movement from the intercellular spaces into the mucosal bathing solution through fluctuating pores in the tight junctions. Curci and Frömter (1979) reported that a $K^+$ concentration of about 3.9 mM exists in the intercellular spaces. This means that the $K^+$ concentration in the interspaces is slightly higher than in the external bathing solutions. Hence, there could exist a driving force for $K^+$ from the interspaces into the mucosal solution. In addition, morphological studies of the tight junctions showed that the existence of ionic pathway with open-close kinetics is possible (Claude, 1978). However, the $K^+$ gradient of about 1.5 mM, existing across the tight junctions (Curci & Frömter, 1979), ought to be abolished by much smaller $K^+$ concentrations than the 36 mM we needed in our experiments to suppress the Lorentzian noise component completely. Hence, the fluctuating structures are probably located in the cell membranes. The asymmetric behavior of the amplitudes of the relaxation noise component, as a function of mucosal and serosal $K^+$ concentration, favors the assumption that the observed fluctuations arise from a $K^+$ efflux at the apical membrane: An increase of mucosal $K^+$ to 36 mM abolished the relaxation noise, probably by decreasing the electrochemical $K^+$ gradient across the apical membrane. On the other hand an increase of serosal $K^+$, which depolarizes both cell membranes (Van Os & Slegers 1975; Reuss, 1979) and probably leads to an increased apical $K^+$ efflux, increases the fluctuations. The conclusion that the fluctuating pores are located in the apical membrane is confirmed by the fact that $TEA^+$ inhibits relaxation noise only when applied from the mucosal side.

It is conceivable that fluctuations, arising at the basolateral membrane could not be detected by our measurements because of the following reasons: (1) The relaxation time of these fluctuations is either to small or too great to be observable in the analyzed frequency range. (2) The amplitudes of these fluctuations are attenuated to such an extent that they could not be detected. The attenuation of possible fluctuations of $K^+$ channels in the basolateral membrane can be estimated. Assuming the values: $R_b=201\ \Omega cm^2$, $R_a=1220\ \Omega cm^2$, $R_p=91\ \Omega cm^2$ (Frömter et al., 1981) and $R_s=90\ \Omega cm^2$ we calculated an attenuation factor of 211 for possible fluctuations originating from basolateral $K^+$ channels. Hence it is possible, that there exist fluctuating channels in the basolateral membrane which could not be detected because of strong attenuation.

*The Shape of the Power Spectra*

In this section possible explanations are given for the $A/f^\alpha$ noise component (low frequency noise) as well as for the deviation between the data points in the power spectra and the fitted Lorentzian curve at higher frequencies.

*a) The Low Frequency Noise Component.* Two distinct types of LF noise could be observed in the gallbladder epithelium. In toad gallbladder, large LF fluctuations, observed under control conditions, could be inhibited by increasing serosal $K^+$ concentration (Van Driessche & Gögelein, 1978). It was assumed that these fluctuations were due to mechanical activities, probably caused by smooth muscle fibers. Similarly, Gordon (1978) demonstrated that smooth muscle contractions in toad urinary bladder caused fluctuations in conductance of the paracellular pathway. In this preparation the smooth muscle contractions could be inhibited by verapamil. A few experiments with toad gallbladders showed that verapamil did not affect the LF noise component *(unpublished results)*. The second type of LF noise could not be inhibited by increasing serosal $K^+$ but its intensity increased strongly, when transepithelial ionic flow was augmented by establishing an ionic concentration gradient. For example, this LF noise increased, when one side of the epithelium was bathed in NaCl-Ringer's and the other side in KCl-Ringer's solution. The LF noise was also present when choline was substituted for $Na^+$ on the mucosal side and the serosal solution consisted of either NaCl- or KCl-Ringer's *(unpublished results)*. This fact shows that the LF noise was not related to a single ion species, but that it was presumably produced by ionic flow through nonselective pathways. As most of the passive transepithelial ionic flow is via the paracellular shunt (Frömter, 1972), it can be assumed that the LF noise is mainly associated with diffusional ionic flow across this pathway. This hypothesis is supported by the fact that all procedures, which reduced cationic permeabilities across the shunt, such as application of $TAP^+$ or $TEA^+$ to the mucosal side or acidification of the mucosal solution, also reduced the LF noise. Noise arising from diffusional processes with $1/f^\alpha$ spectrum, was observed in artificial systems (Dorset & Fishman, 1975; Green, 1976) as well as in excitable tissues (Conti, DeFelice & Wanke, 1975; Fishman, Moore & Poussart 1975). However, in most reported cases the slope $\alpha$ is smaller than 2. As $\alpha$ is greater than 2 in the gallbladder epithelium, the LF noise may be of other origin. For example it is possible that it arises from permeability changes of cell membranes and/or in the paracellular pathway, which causes fluctuations of ionic current across these structures.

*b) The Noise Component at Frequencies above 10 Hz.* As pointed out, good fits with a single Lorentzian were possible only by excluding data point above 10 Hz. However, power spectra could be adequately fitted by the superposition of two Lorentzians. Calculations showed that such deviations from a single Lorentzian cannot be explained by a frequency dependent attenuation effect (Van Driessche & Gögelein, 1980). In the following paper additional evidence will be given that the power spectra in *Necturus* gallbladder contain two Lorentzian components. Possible underlying mechanisms are discussed there.

*Effects of $TAP^+$*

It was shown that $TAP^+$ decreases cationic conductance of the paracellular pathway of the gallbladder epithelium (Moreno, 1975; Reuss & Grady, 1979). This increase in $R_p$ reduces the attenuation of $K^+$ fluctuations, arising in the apical membrane (Van Driessche & Gögelein, 1980). Under the assumption that $TAP^+$ increases only $R_p$ and has no effect on other epithelial structures, the effect of $TAP^+$ on current and voltage fluctuations can be estimated with Eqs. (3) and (4). We calculated the square of the ratio of the current fluctuations with and without $TAP^+$: $Q_I=(\Delta I(\text{TAP})/\Delta I(\text{no TAP}))^2$ and the corresponding ratio for voltage noise $Q_V=(\Delta V(\text{TAP})/\Delta V(\text{no TAP}))^2$. With the parameters $R_a=4505$, $R_b=2750$ *(see above)*, $R_s=90$, $R_p(\text{no TAP})=90$ and $R_p(\text{TAP})=200$ *(own observations)* we obtain $Q_I=2.1$ and $Q_V=5.9$. This means that after

addition of $TAP^+$, current fluctuations should increase by the factor 2.1 and voltage fluctuations by the factor 5.9. In experiments with *Necturus* gallbladder, however, the plateau value $S_o$, obtained from current noise spectra, decreased (Table 1), whereas in voltage noise spectra the Lorentzian component increased after application of $TAP^+$ (*see* Fig. 4). This increase in voltage fluctuations was roughly estimated by comparing spectral values at 10 Hz ($S_V$(10 Hz)) before and after application of $TAP^+$. About 15 min after administration of $TAP^+$, $S_V$(10 Hz) increased by the factor of $2.7 \pm 0.6$ ($N=8$). This value is about half of the increase predicted by the attenuation effect. This discrepancy, and the fact that the current fluctuations are depressed, instead of being increased by $TAP^+$, shows that $TAP^+$ decreases $K^+$ conductance fluctuations. This is in agreement with results of Reuss and Grady (1979), who reported a decrease of apical membrane $K^+$ conductance after application of $TAP^+$.

*The Pore Model*

The simplest conceivable pore model consists of randomly fluctuating channels with a single conducting state. As we do not know the transition probabilities for transitions between the open and closed state, we assumed the mean time open and the mean time closed to be equal. In such a model the current $i_K$ through an open pore and the total number of pores $M$ is given by (Verveen & DeFelice, 1974; Lindemann & Van Driessche, 1977):

$$i_K = S_o/(2 \cdot I_K \cdot \tau) \quad (5)$$

$$M = 2 \cdot I_K/i_K \quad (6)$$

where $\tau$ is the relaxation time given by

$$\tau = 1/(2 \cdot \pi \cdot f_c) \quad (7)$$

and $I_K$ is the mean $K^+$ current through the apical membrane.

Single-channel parameters were estimated with data recorded with NaCl-Ringer's on both sides (Table 1, row 1). The Lorentzian component under these conditions is assumed to be due to an apical $K^+$ efflux. The driving force for this efflux $V_K$ is reported to be 32.3 mV (Reuss & Weinman, 1979). With the apical $K^+$ resistance of $R_a = 4505\ \Omega cm^2$, estimated above, one calculates $I_K = 7.2\ \mu A/cm^2$. Correcting the plateau value $S_o$ of Table 1 (row 1) with the attenuation factor $A = 11.85$ yields $S_{intr} = 9.2 \times 10^{-19}\ A^2 sec/cm^2$. Inserting these values, as well as $\tau = 49.7$ msec (Table 1, row 1) into Eqs. (5) and (6) yields $i_K = 1.3$ pA and $M = 0.11\ \mu m^{-2}$. The single-channel conductance calculated from $g_K = i_K/V_K$ is $634\ \mu m^2$ (Spring & Hope, 1979) we calculate a density of about 70 channels per cell. Performing the same estimation with the values $R_a = 1220\ \Omega cm^2$, $R_b = 201\ \Omega cm^2$ and $R_p = 91\ \Omega cm^2$ (Frömter et al., 1981), while all other parameters remain unchanged, yields $g_K = 6.5$ pS and $M = 1.9$ pores per $\mu m^2$. This rough estimation of the single-channel conductance is in the same order of magnitude as that reported for node of *Ranvier* ($g_K = 2.9$ pS) (Van den Berg, Siebenga & De Bruin, 1977) or the squid axon membrane ($g_K = 12$ pS) (Conti et al., 1975). Further common properties between apical $K^+$ channels in *Necturus* gallbladder and in excitable tissues is their inhibition by $TEA^+$ (Conti et al., 1975; Fishman et al., 1975) and protons (Drouin & The, 1969; Hille, 1973), as well as the permeability sequence $K^+ > Rb^+ > Cs^+$ (Hagiwara, Eaton, Stuart & Rosenthal, 1972; Hille, 1973). The inhibition of the $K^+$ channels by protons as well as the mentioned permeability sequence could be due to the existence of acidic sites inside the channels.

The similarities with respect to the effect of $TEA^+$ supports the assumption of the pore model which was postulated by Armstrong (1975) for the $K^+$ channel in nerve membranes. He concluded that the pore consists of a mouth with a diameter of about 8 Å and a narrow part ("tunnel") with a diameter of about 3 Å. The mouth is wide enough to accept $TEA^+$ or partly hydrated $K^+$ ions. In order to penetrate the tunnel the $K^+$ ions must lose their hydration shell, whereas $TEA^+$ ions cannot enter the narrow part but can prevent the passage of other ions.

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