# FLUCTUATION ANALYSIS OF APICAL K CURRENTS IN THE URINARY BLADDER OF THE WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS).

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Active K transport by the urinary bladder of the winter flounder (Pseudopleuronectes americanus) can be measured as a short circuit current. $I_K$ is reversibly blocked by barium added to the mucosal bath (Bull. M.D.I.B.L. 20:84, 1982) suggesting that the apical membranes of the K-transporting cells contain a population of K channels which are reversibly blocked by barium and which mediate conductive K exit from the cells to the lumenal bath. The object of the present study was to obtain more direct evidence for the presence of apical K channels in the flounder urinary bladder by analyzing fluctuations or "noise" in the transmural current. Previous studies using other epithelial cell layers have shown that an analysis of the fluctuations in macroscopic currents due to Na (Lindemann and Van Driessche, Science 195:292, 1977) or K (Van Driessche and Gogelein, Nature 275:665, 1978) can provide information about the properties of the underlying single channel events. The analysis of current fluctuations is based on a model which is used to relate the gating of a single channel to the fluctuations in macroscopic current observed due to the stochastic properties of a population of identical, independent channels. Gating refers to the transitions between the conducting (open) and non-conducting (closed) states. These transitions may be "spontaneous", i.e. occuring in the absence of agonists of blockers or "induced" by the addition of a reversible blocker. An analysis of the fluctuations in transmural K currents in flounder urinary bladder revealed a component of "spontaneous" noise which we have tenatively attributed to the apical K channels. Additional components of noise could be induced by either barium or lidocaine, both of which are blockers of K transport in this tissue.

Bladders were removed from flounder, glued as flat sheets onto plastic rings and mounted in a perfusion chamber similar to that described by Abramchek, Van Driessche and Helman, (J. Gen. Physiol. 85:555, 1985). Both sides of the tissue were continuously perfused with solutions containing (in mM) Na: 147.5, Cl: 147.5, K: 2.5, Ca: 1.5, Mg: 1.0, HEPES: 15.0, and glucose: 10.0. The serosal solution also contained verapamil (50 µM) to inhibit the contraction of smooth muscle. The pH of the solution was 7.5.

Methods for acquiring and analyzing K channel noise were similar to those described in detail by Helman, Cox and Van Driessche (J. Gen. Physiol. 82:201, 1983). In brief, the tissues were voltage-clamped by means of a specially designed, low-noise circuit. The signal was filtered to remove the D.C. component and unwanted A.C. components, further amplified and then digitized. Twenty to thirty time domain sweeps were accumulated at sampling rates of 2 ms and each sweep was transformed to the frequency domain (FFT) for subsequent calculation of the current-noise power density spectrum (PDS).

The analysis of the PDS was based on a comparison of the observed spectrum with that predicted on the basis of the simplest model for single channel gating: a homogeneous population of channels, each member of which exhibits transitions between a conducting (open) and non-conducting (closed) state. These transitions are presumed to occur randomly, but over time they are presumed to be consistent with a set of probabilities for finding the channel in the conducting or non-conducting state. The values of these probabilities are related to the values of the rate coefficients which characterize the reversible transition,

$$\text{conducting} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \text{non-conducting}$$

The PDS is a plot of the log of the spectral intensity, S(f) vs. the log of the frequency. The spectral intensity (in $A^2 \cdot sec/cm^2$) may be thought of as the power dissipated by the noise current if it passed through a one ohm resistor. The spectrum predicted for a simple, on-off channel event is characterized by a low frequency plateau. As frequency increases the spectral intensity is constant until the "corner frequency", $f_c$, is reached after which the spectral intensity falls off rapidly with frequency. A spectrum of this form is referred to as a "Lorentzian" type and is characteristic of a single, two-state process. The identification of a Lorentzian component in the PDS is generally taken as evidence that the observed noise is due in part to single-channel activity. The corner frequency, $f_c$, is related to the rate coefficients for channel opening and closing:

$$2\pi f_c = \alpha + \beta$$

An intuitive impression of the significance of $f_c$ can be obtained by considering a case in which $\alpha$ (the rate coefficient for closing) is much greater than the rate coefficient for opening ($\beta$). In this condition the corner frequency is equal to the inverse of the mean open time for the channel.

PDS were measured as described above in the presence and in the absence of blockers of active K secretion. Figures one and two illustrate results obtained in one such experiment. Figure one shows a spectrum obtained under control conditions in the absence of blockers. Inspection of the data points revealed a distinct Lorentzian component in the spectrum. The Lorentzian component was extracted by fitting the points to an equation which was the sum of a single Lorentzian (channel noise) component and a component of 1/f ("background") noise. The solid lines show the results of the fit. Figure two shows a spectrum recorded in the presence of 3mM mucosal $BaCl_2$. Again the solid lines show the non-linear, least squares fit to the sum of a single Lorentzian component and a component of 1/f noise. A comparison of figures one and two suggests that mucosal barium shifted the corner frequency to lower values. This is consistent with the notion that the control spectra was dominated by spontaneous gating of apical K channels while the spectrum in the presence of barium was dominated by fluctuations induced due to reversible blockade of K channels by the divalent ion. Lidocaine reduced $I_K$ and also shifted the corner frequency of the PDS (not shown).

The results presented here are consistent with the notion that the apical membranes of flounder urinary bladder cells contain a population of K channels which are reversibly blocked by barium or lidocaine, and suggest that blocker-induced fluctuations in apical K currents may provide a method for estimating the value of the single channel currents and the number of K channels under different experimental conditions.
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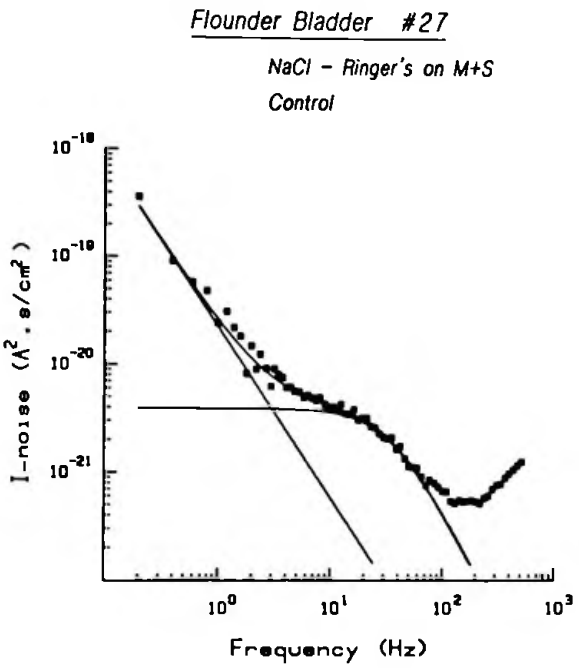


Figure 1

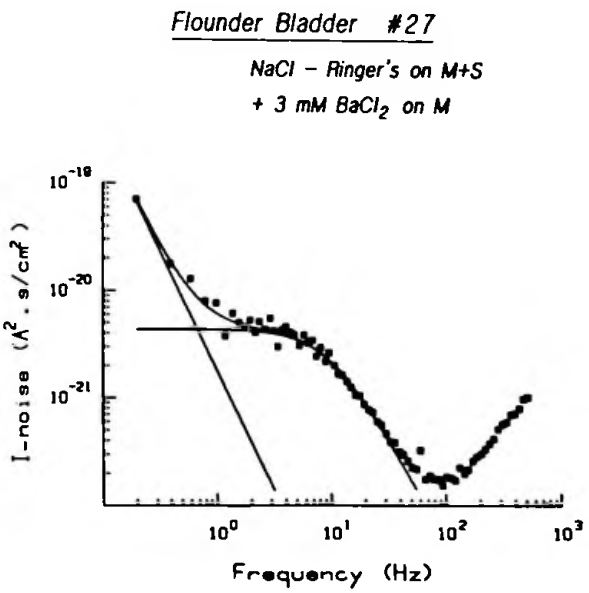


Figure 2