Biochimica et Biophysica Acta, 732 (1983) 636–646
Elsevier

BBA 71771

COMPETITIVE BLOCKING OF APICAL SODIUM CHANNELS IN EPITHELIA

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(Received March 9th, 1983)

Key words: Noise analysis; Na$^+$ channel; Epithelial membrane; Open-closed kinetics; Amiloride; Triamterene; (Frog skin)

Apical sodium-selective channels in frog skin, when blocked by amiloride or triamterene, exhibit fluctuations in current, the spectra of which are Lorentzian. These effects have been modeled previously with two-state and three-state models by Lindemann and Van Driessche. A recent observation by Hoshiko and Van Driessche that corner frequencies are lowered by increasing the apical sodium concentration cannot be accounted for by these models. We explore the possibility that sodium (S) and amiloride (A) compete for a site at the mouth of the channel. A new three-state channel model (sodium-occupied, open/unoccupied, open/amiloride-blocked) is analyzed. Its corner frequency is of the form $f_c = f_{c0}[1 + (A/K_A)/(1 + S/K_S)]$, consistent with the observed sodium dependence of the corner frequency. The minimum frequency, f_{c0}, and the inhibition constants, K_A and K_S, are expressed in terms of the rate constants of the model. To account for sodium self-inhibition, we postulate that two sodium ions in the channel may result in clogging – a fourth state. The two corner frequencies are calculated; so are the plateau values of the noise power. The noise power shows a maximum as a function of blocker concentration, as observed previously using triamterene. The four-state model predicts the observed suppression by small amounts of blocker of the low-frequency sodium (clogging) noise.

In epithelial membranes such as frog skin and toad bladder, transepithelial sodium transport is modulated primarily by changes in the sodium permeability of the apical surface membrane of the epithelium. This was suggested by the early demonstration [1] that over 80% of the transepithelial resistance is located at the apical surface. Apical sodium permeability can be blocked by various agents. Amiloride and triamterene are of particular interest as blockers because of their high specificity in inhibiting apical sodium permeability [2–4]. Cuthbert and Shum [5] have compiled evidence that amiloride binding is associated with sodium channels and that amiloride and sodium compete for binding sites. Since the apparent amiloride inhibition constant increases with sodium concentration, they suggest that amiloride acts by competitive inhibition with sodium ion. Others, like Benos et al. [6], found evidence for non-competitive inhibition in the bullfrog, *Rana catesbeiana.* Sodium transport also shows self-inhibition, evident in the saturating behavior of sodium flux as a function of sodium concentration in the outside bathing solution [7].

Early interpretations were made in terms of a carrier as the mechanism of ion translocation across frog skin [8]. More recent evidence points to a channel mechanism. (1) Lindemann and Van Driessche [9] calculated the single-channel current from alimoride-induced current fluctuations in frog skin and concluded that the carrier turnover frequency required to account for that current

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would be too high. They chose to study amiloride-induced noise because it exhibits relatively high characteristic frequency (1–30 Hz) compared to sodium self-inhibition, whose characteristic frequency must be well below 1 Hz [10]. (2) Amiloride- and triamterene-induced current noise power was found to be proportional to the squre of the mean current [11], as would be expected for a channel mechanism. A carrier mechanism would give a first-power dependence, according to the classical shot-noise formula. These results were interpreted in terms of a simple two-state channel model. Van Driessche and Lindemann [12] also found that the sodium self-inhibition effect was ascribable to a decrease in the number of available channels with increasing sodium concentration, and that the amiloride-blocking rate constant was independent of the apical sodium concentration when the corium surface was bathed in potassium chloride solution.

In recent noise measurements [13], the rate constant for triamterene blocking of the sodium channel was found to decrease with increasing sodium concentration when sodium was present in the corium bathing solution. Similar results have been obtained for amiloride *. While such a sodium dependence is consistent with the results of steady-state measurements [3], it cannot be explained by the three-state model of Lindemann and Van Driessche [10]. In Section 5 it is shown that such a model, with two closed states and one open state, predicts the wrong sign for the sodium dependence. In that model, the blocking molecule can attach itself to any open channel, whether that channel is empty or contains sodium. The observed sodium suppression of the blocking rate strongly suggests that the blocker molecules and the sodium ions compete for binding sites. A simple possibility is that once a sodium ion has entered a channel, entrance to that channel is forbidden to the blocking molecule until the sodium has moved either forward along the channel or back into the apical bathing solution. This paper analyzes two simple models involving such competitive inhibition: a three-state model for the amiloride blocking, and a four-state model to include sodium self-inhibition. The four-state model turns out to predict the observed suppression of the low-frequency sodium noise by the addition of small amounts of blocking agent (Hoshiko and Van Driessche, unpublished).

A preliminary account of this work was given at the 1982 Annual Meeting of the Biophysical Society [31].

(I) Corner frequency

One may picture the sodium-selective channel as a single-file pore [14–19] or as some sort of opening with two or more energetically different sodium binding sites near its mouth (Rick, R., personal communication). An adequate model must explain noise in the 0.1–100 Hz range associated with the blocking molecule (e.g., amiloride or triamterene) as well as the lower-frequency noise associated with sodium inhibition of the sodium channels. Although it cannot account for sodium competition, we first review the simplest two-state model. We then proceed to multi-state models which take sodium competition into account.

(1) Blockable sodium channel – simplest model

The blocking-unblocking alone leads naturally to a two-state model [20] and to noise with a Lorentzian spectrum [21]. Write the subscripts 'o' for open and 'A' for amiloride-blocked for the two states of the channel. Their respective probabilities, R_o and R_A, sum to unity:

$$R_o + R_A = 1$$

That neglects sodium self-inhibition, which we shall call clogging and discuss below. Let A equal the amiloride concentration and name the rate constants according to the scheme

$$R_o \underset{k_-}{\overset{kA}{\rightleftarrows}} R_A \tag{1}$$

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* Hoshiko and Van Driessche, personal communication. Analysis of variance of the triamterene data shows that the sodium effect on the association rate constant is significant at the 5% level. Sodium effects on the low-frequency plateau value and on the single-channel current just miss significance.

Then we have the rate equations

$$\mathrm{d}R_0/\mathrm{d}t = -kAR_0 + k_- R_A$$

$$\mathrm{d}R_A/\mathrm{d}t = kAR_0 - k_- R_A \tag{1'}$$

They have decaying exponential solutions with decay rate

$$\omega_c = k_- + kA \tag{2}$$

Accordingly, the autocorrelation function of the sodium current is $\exp(-\omega_c t)$. The power spectrum is proportional to the Fourier transform of this autocorrelation and has the familiar Lorentzian form

$$1/(1+\omega^2/\omega_c^2)$$

with corner frequency $f_c = \omega_c/2\pi$. The corner ω_c is the sum of the rate k_- at which the closed state opens ($= 1/\tau$ of Ref. 21) plus the rate kA at which the open state closes ($= 1/\sigma$ of Ref. 21). The linear dependence of the corner frequency on amiloride concentration was shown by Lindemann and Van Driessche [9]. The slope of this function (Eqn. 2) is k and the intercept is k_-.

Note that the R_i terms are formulated as probabilities rather than, perhaps more conventionally, as numbers of channels. This normalization simplifies the notation for the three- and four-state models.

(2) Three-state model – two states open, one state closed

To obtain a dependence of corner frequency on sodium concentration, S, we go to a model in which we distinguish whether the open channel is empty or has a sodium ion in it. Call the probabilities R_0 and R_1, respectively. Again the three probabilities sum to unity:

$$R_0 + R_A + R_1 = 1$$

The crucial assumption is that the blocking is competitive, i.e., that if there is a sodium ion in the channel, the blocking is prevented. The simplest reaction scheme incorporating this competitive inhibition is:

$$R_1 \underset{k_{-1}}{\overset{k_1 S}{\leftrightarrows}} R_0 \underset{k_-}{\overset{kA}{\rightleftarrows}} R_A \tag{3}$$

The rate (probability per unit time) at which sodium ions (concentration S) enter the empty channel is $k_1 S$. The rate at which blocker molecules (concentration A) enter the empty channel is kA. The respective emptying rates, k_{-1} and k_-, are concentration-independent. Note that the sodium turnover rates are in the megahertz range, the blocker turnover rates of the order of 10 Hz (see Fig. 1). The rate equations governing the time-course of the three probabilities are:

$$\begin{aligned}
\mathrm{d}R_0/\mathrm{d}t &= -kAR_0 + k_- R_A \quad -k_1 SR_0 + k_{-1}R_1\\
\mathrm{d}R_A/\mathrm{d}t &= kAR_0 - k_- R_A\\
\mathrm{d}R_1/\mathrm{d}t &= \qquad\qquad\qquad\quad\; k_1 SR_0 - k_{-1}R_1
\end{aligned} \tag{4}$$

The fluctuations due to blocking are again associated with the closing and opening of the channels. Their spectrum will consist of a sum of two Lorentzians. One might guess that the two corners would be approximately $k_- + kA$ and $k_{-1} + k_1 S$, respectively. Those are the round-trip rates of the individual steps of the reaction scheme – 'uncoupled' eigenvalues. The guess has to be modified to take account of the competition. In Section 5 below we show how the 'blocking' corner depends on sodium concentration. If $k_- \ll k_{-1}$, i.e., if sodium moves out of the channel much more quickly than blocker, the corner is given approximately by

$$2\pi f_c = \omega_{\text{blocking}} = k_- + kA/(1 + k_1 S/k_{-1}) \tag{5}$$

This increases linearly with blocker concentration A. The slope depends on sodium concentration in

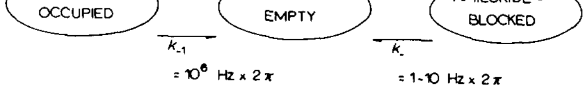

Fig. 1. Reaction scheme for the three-state model with competitive inhibition. Amiloride blocker molecule in the channel prevents entry of sodium; sodium ion in the channel prevents blocking.

classical inhibition form. In other words, the model shows the observed [13] sodium depression of the blocking corner frequency. It also agrees with the observation [9,13] that the intercept, i.e., the extrapolated zero-blocker ($A \rightarrow 0$) limit, is sodium-independent.

The model gives the expected 'inhibition' behavior for the steady-state current, allowing comparison with short-circuit current measurements. Since states 'o' and '1' are both open-channel states, the current through one channel can be written (see Section III, 'Critique')

$$I_{DC} = i_o(R_o^s + R_1^s),$$

where the superscript 's' means 'steady-state' ($dR/dt = 0$). This has the same sodium dependence as the blocking term in Eqn. 5:

$$I_{DC} = i_o/[1 + kA/k_-(1 + k_1S/k_{-1})]$$

The blocker concentration giving 50% current inhibition is reported [3] to increase with sodium concentration, in agreement with this equation. From the DC measurements of Cuthbert and Shum (Ref. 3, Fig. 3) the constant k_{-1}/k_1 is about 6 mM of sodium, consistent with the noise data of Hoshiko and Van Driessche (unpublished data). According to the model, this constant should be independent of the blocker used.

(3) The other eigenvalue due to shot effect

The discrete nature of the charge carried by sodium gives rise to shot noise, the corner frequency of which is well above that due to blocker action and too high to be observed in practice. For single-channel currents of 0.4 pA found by Lindemann and Van Driessche [9] about 3 000 000 sodium ions transit through the pore per second. Each ion jump represents a current pulse lasting only a fraction of a microsecond, corresponding to a corner frequency in the megahertz region. Not only can the usual amplifiers not respond at such frequencies; it is actually necessary to filter out frequencies above approx. 1 kHz to eliminate the artifact due to the input voltage noise of the clamp amplifier [24]. So the current pulses due to the passage of individual ions merge to give an apparently constant current. In the presence of a blocker, the steady current is turned on and off with characteristic times of tens or hundreds of milliseconds. It is this lower-frequency noise associated with the blocking and unblocking that is measured.

To be sure, there will be a high-frequency 'corner' in the megahertz range for the 'true' spectrum of the current noise, as distinct from the measured spectrum. The three-state model described above also does not show this true shot noise, which is white up to megahertz frequencies. Of course, a sodium ion entering the channel makes a contribution to the forward current. But the 'o' → '1' transition that represents it in the present model is noiseless, since it is an open-open transition. What it does is to take the channel from a blockable to a non-blockable state. Increasing the sodium concentration inhibits the blocking and actually cuts down the level of the blocking noise. This is shown in Section 5 below. It should be remarked that a '1' → 'o' transition in which the sodium ion goes forward through the pore, contributing to direct current (DC), is indistinguishable in our model from one in which the sodium ion comes back out into the apical bathing solution, resulting in no DC contribution.

The more detailed models which specify the occupation state of two or more sites in the channel [25,17] do show the shot noise, which has either a falling or a rising Lorentzian tail at the frequency characterizing the rate at which an ion moves into and out of the channel. The model of Frehland's [25] (Fig. 2) becomes otherwise equiva-

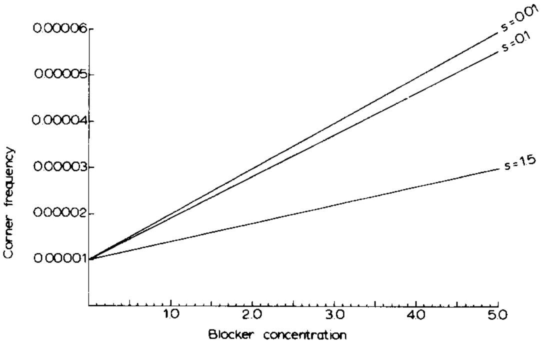

Fig. 2. Corner frequency ω_{1o} for the three-state model as a function of blocker concentration for two different sodium concentrations, according to Eqn. 9, Rate constants are $k_{-1} = 1$ Hz × 2π, $k_- = 0.00001$ Hz × 2π, so that $\xi = k_-/k_{-1} = 0.00001$. Blocker concentration is expressed in dimensionless units (Eqn. 7).

lent to the present model upon eliminating his state '3'.

(4) The noise spectrum of a three-state model is the sum of two Lorentzians

The sophisticated reader will say, "Of course! Linear models with real eigenvalues have Lorentzian spectra". A linear Markoff process has a Lorentz $= (1 + \omega^2/\omega_c^2)^{-1}$ spectrum, flat at low frequencies, inverse-square at frequencies much higher than the corner ω_c. Here is a concise proof.

The three rate equations (Eqn. 4) describing the three-state model are not linearly independent. Any one of the three variables can be eliminated using the normalization condition $R_o + R_A + R_1 = 1$, which expresses the fact that the R_i terms are probabilities. The solution for the two variables remaining is a superposition of decaying exponentials. This solution describes the average relaxation of the system from any initial state to the steady state. The autocorrelation (and the regression) function for each variable has this same time behavior. The noise spectrum is proportional to the Fourier transform of the autocorrelation function. Recall that the Fourier transform of an exponential is a Lorentzian, and the proof is complete. Indeed, the two corners ω_{lo} and ω_{hi} are the eigenvalues of the 2×2 coefficient matrix of the system of linear differential equations [26]. It is straightforward to prove that these eigenvalues are indeed real, i.e., that a three-state model has no oscillatory solutions.

In Chen's [26] matrix notation, the differential equations (Eqn. 4) are written

$$-\mathrm{d}\boldsymbol{R}/\mathrm{d}t = \boldsymbol{A}\boldsymbol{R} \tag{6}$$

with the rate-constant matrix

$$\boldsymbol{A} = k_{-1}\begin{pmatrix} s + \xi a & -\xi & -1 \\ -\xi a & \xi & 0 \\ -s & 0 & 1 \end{pmatrix}$$

expressed in terms of the dimensionless parameters *

$$a = kA/k_-,\ s = k_1 S/k_{-1},\ \xi = k_-/k_{-1}. \tag{7}$$

To write this in two linearly independent variables, one defines a 2×2 matrix $A'_{ij} = A_{ij} - A_{i0}$, where the subscript zero refers to the variable being suppressed. If, for example, we choose to keep R_o and R_A, suppressing R_1, then we have

$$\boldsymbol{A}' = k_{-1}\begin{pmatrix} 1 + s + \xi a & 1 - \xi \\ -\xi a & \xi \end{pmatrix} \tag{8}$$

Its eigenvalues are the two corners, ω_{lo} and ω_{hi}:

$$\omega_{\mathrm{lo}}^{\mathrm{hi}} = k_{-1}\Big\{\tfrac{1}{2}(1 + s + \xi + \xi a) \pm \Big[\tfrac{1}{4}(1 + s + \xi + \xi a)^2 - \xi(1 + a + s)\Big]^{1/2}\Big\} \tag{9}$$

The 'blocking corner' ω_{lo} is plotted in Fig. 2.

(5) Sodium dependence of ω_{lo}: derivation of Eqn. 5

If state '1' is suppressed (limit $S \to 0$) the single corner is $\omega_c = k_- + kA$, in agreement with Section 1 above. To study the dependence of ω_{lo} on sodium concentration, S, we expand in powers of the dimensionless ratio $\xi = k_-/k_{-1}$ of the two dissociation rate constants. Because ξ is so small ($\approx 10^{-5}$), an expansion will converge rapidly. Defining another small dimensionless variable, ϵ, by

$$\omega_{lo} = k_- + \epsilon k_{-1} \tag{10}$$

the eigenvalue equation can be written in determinantal form,

$$\begin{vmatrix} 1 + s + \xi a - \xi - \epsilon & 1 - \xi \\ -\xi a & -\epsilon \end{vmatrix} = 0 \tag{11}$$

Dropping terms of order higher than the first in ξ and ϵ, we have

$$\epsilon = \xi a/(1 + s)$$

The result for the lower-frequency eigenvalue (the 'blocking' corner) is

$$\omega_{\mathrm{lo}} = k_-[1 + a/(1 + s)] \tag{5}$$

as announced in Section 2. The small-ξ approximation for the higher eigenvalue gives

$$\omega_{\mathrm{hi}} = k_{-1}[1 + s + \xi as/(1 + s)] \tag{12}$$

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* A glance at the rate equations (Eqn. 4) will give an interpretation of a and s as ratios of the steady-state probabilities R_i^s:

$$a = R_A^s/R_o^s,\ s = R_1^s/R_o^s,$$

so that

$$R_o^s = 1/(1 + a + s),\ R_A^s = a/(1 + a + s),\ R_1^s = s/(1 + a + s).$$

correct to first order in ξ. Our 'first guess', $\omega_{\mathrm{hi}} = k_{-1} + k_1 S$, the 'uncoupled' round-trip o → 1 → o rate, constitutes the first two terms.

The method of this section can also be applied to the three-state model of Lindemann and Van Driessche [10]. Formally, that model is given by the reaction scheme of Eqn. 3, but the interpretation is quite different. There the one-sodium state is a closed-channel state, postulated in order to explain sodium self-inhibition. Accordingly, the dissociation rate constant for the one-sodium-to-zero-sodium transition would be very slow. Indeed, the absence of a corner in the 'sodium noise' spectrum down to frequencies as low as 0.1 Hz indicates that this dissociation rate is much slower than k_-; i.e., $\xi \gg 1$. The blocking noise corner thus represents the higher eigenvalue. Differentiating Eqn. 9 with respect to sodium concentration, s, shows that this eigenvalue increases with increasing sodium concentration. One concludes that a three-state model with two closed states cannot explain the observed sodium-dependence of the blocking corner.

We do, however, want to model the sodium noise (current fluctuations in the absence of blocker), which is presumably associated with sodium self-inhibition. Some sort of sodium clogging has to be postulated. This is done in the next section.

(6) *A four-state model – blocking and clogging*

The mechanism of sodium clogging is not yet clear. It may involve some cooperative phenomenon. Perhaps the simplest picture is that the presence of two sodium ions in one channel triggers a conformational change of the channel, closing it off to further sodium transport. Any two-sodium collective phenomenon that chokes off the channel results in the same 'reaction scheme' (see Eqn. 13). Observed low-frequency noise power in the sodium current in the absence of blocker has an inverse-square frequency dependence, consistent with a Lorentzian component with corner frequency well below 1 Hz [27]. The reopening of the channel may be considered to be thermally activated, with an activation energy of several tenths of an electron-volt. Another possibility is that near the channel mouth there are two sodium-specific sites so situated that one site is accessible to a sodium ion only if the other site is already occupied. The following analysis applies to either possibility.

Let the collapsed or clogged state '2' have probability R_2, so that all four probabilities sum to 100%:

$$R_{\mathrm{o}} + R_{\mathrm{A}} + R_1 + R_2 = 1$$

The reaction scheme is:

$$R_2 \underset{k_{-2}}{\overset{k_2 S}{\leftrightarrows}} R_1 \underset{k_{-1}}{\overset{k_1 S}{\leftrightarrows}} R_{\mathrm{o}} \underset{k_-}{\overset{kA}{\rightleftarrows}} R_{\mathrm{A}} \tag{13}$$

Note that both 'o' and '1' are open states, both 'A' (= blocked) and '2' (= clogged) are closed states. The rate-constant matrix is

$$\boldsymbol{A} = \begin{pmatrix} kA + k_1 S & -k_- & -k_{-1} & 0 \\ -kA & k_- & 0 & 0 \\ -k_1 S & 0 & k_{-1} + k_2 S & -k_{-2} \\ 0 & 0 & -k_2 S & k_{-2} \end{pmatrix} \tag{14}$$

The rate constants k_1, k_{-1}, k_2 and k_{-2} should surely be independent of which blocker is used, unlike k and k_-. Again, transitions between states 'o' and '1' are responsible for the direct current, but are too fast to be resolved. The measured noise comes from o ⇆ A transitions (blocking and unblocking) and 1 ⇄ 2 transitions (clogging and unclogging). Judging from frog skin data, the rate constant k_{-2} is smaller than k_-; i.e., the clogging noise ('sodium noise') is at much lower frequencies than the blocking noise. The 'true' noise spectrum will now consist of three Lorentzians. The highest corner frequency (largest eigenvalue of $\boldsymbol{A}'$) is surely in the megahertz range (frequency range starting at $k_{-1}/2\pi$). The intermediate corner frequency $\omega_{\mathrm{blocking}}/2\pi$ is in a range starting at $k_-/2\pi$. The lowest corner frequency $\omega_{\mathrm{clogging}}/2\pi$ is in a frequency range starting at $k_{-2}/2\pi$.

To see how these corners depend on sodium concentration, S, and on blocker concentration, A, expand in powers of small dimensionless variables

$$\eta = k_{-2}/k_- \text{ and } \zeta = k_2 k_{-1}/k_1 k_- \tag{15}$$

defined so that the equilibrium constant between states '2' and '1' is $k_2 S/k_{-2} = \zeta s/\eta$. To study $\omega_{\mathrm{clogging}}$ we define a dimensionless variable, ϵ, by

$$\omega_{\mathrm{clogging}} = k_{-2} + k_- \epsilon \tag{16}$$

or

$$\omega_{\text{clogging}}/k_{-1} = \xi(\eta + \epsilon)$$

knowing that ϵ will be small. The eigenvalue equation, in determinantal form, is

$$\begin{vmatrix} s + \xi a + \xi - \xi\eta - \xi\epsilon & -1 + \xi & \xi \\ -s & 1 - \xi\eta + \xi\zeta s - \xi\epsilon & -\xi\eta \\ 0 & -\xi\zeta s & -\xi\epsilon \end{vmatrix} = 0 \qquad (17)$$

But this cubic equation in ϵ poses no threat. We drop terms of third and higher order in ξ, η and ζ. In units of k_{-1}, the eigenvalue ω_{clogging} comes out

$$\omega_{\text{clogging}}/k_{-1} = \xi\eta + \xi\zeta s^2/(1 + a + s) \qquad (18)$$

which is just a shorthand way of writing

$$\omega_{\text{clogging}} = k_{-2} + k_2 S(k_1 S/k_{-1})/(1 + kA/k_{-} + k_1 S/k_{-1}) \qquad (18')$$

Our first guess might have been $k_{-2} + k_2 S =$ opening rate + clogging rate. The eigenvalue is less than this guess, again because of competition. Note the 'classical inhibition' shape and the analogy to Section 2.

Comparison with experiment is elusive here, since no corner has been observed in the 'sodium noise' spectrum, even though measurements have been made below 0.1 Hz. On the other hand, transients in the short-circuit current following sudden changes in sodium concentration [28] show some relaxation times of the order of 1–3 s. To explain why these do not show up as corners in the noise spectra, one is led to postulate a highly nonlinear mechanism.

In the presence of clogging, the 'blocking' corner differs from the value $k_{-1}\xi[1 + a/(1 + s)]$ derived earlier only by a second-order term.

(II) Noise power

How does the noise level depend on the blocker concentration, A, and on the sodium concentration S? Since the corner frequency of the Lorentzian spectrum (Section 4) depends on both concentrations, it is sufficient to ask the question for the low-frequency plateau of the Lorentzian, i.e., the limit $\omega \to 0$. The salient feature of the A-dependence comes out even in the two-state model, or, which is the same thing, the zero-sodium limit:

$$R_{\text{o}} \underset{k_{-}}{\overset{kA}{\rightleftarrows}} R_{\text{A}} \qquad (1)$$

Eqns. 1′ and 2, $\omega_{\text{c}} = k_{-} + kA = k_{-}(1 + a)$, give the corner frequency, i.e., the characteristic rate constant for the decay of the autocorrelation $e^{-\omega_{\text{c}} t}$. The steady-state probability that the channel be open is $R_{\text{o}}^{\text{s}} = k_{-}/(k_{-} + kA)$. If current i_{o} flows in the open (unblocked, state 'o') channel, the variance of the single-channel current is given by the Bernoulli formula

$$\begin{aligned} \sigma_{\text{I}}^2 &= i_{\text{o}}^2 R_{\text{o}}^{\text{s}} R_{\text{A}}^{\text{s}} \\ &= i_{\text{o}}^2 k_{-} kA/(k_{-} + kA)^2 \\ &= i_{\text{o}}^2 a/(1 + a)^2 \end{aligned} \qquad (21)$$

where $R_{\text{o}}^{\text{s}} = 1/(1 + a)$ and $1 - R_{\text{o}}^{\text{s}} = R_{\text{A}} = a/(1 + a)$ are the steady-state probabilities of the channel being open and closed, respectively. The autocovariance of the current is $\xi_{\text{I}}^2 e^{-\omega_{\text{c}} t}$. Its Fourier transform is the power spectrum of the current:

$$\begin{aligned} G(\omega) &= \frac{1}{\pi} \frac{\sigma_{\text{I}}^2}{\omega_{\text{c}}} \frac{1}{1 + \omega^2/\omega_{\text{c}}^2} \\ &= \frac{1}{\pi} \frac{i_{\text{o}}^2}{k_{-}} \frac{a}{(1 + a)^3} \frac{1}{1 + \omega^2/\omega_{\text{c}}^2} \end{aligned} \qquad (22)$$

Evidently there is little noise power for small values of the blocker concentration, because the channels are usually open. Also there is little noise power for large blocker concentrations, because the channels are usually closed. There has to be a maximum. The maximum in the low-frequency plateau is obtained by setting the derivative of $a/(1 + a)^3$ equal to zero. It occurs at $a = \frac{1}{2}$, or blocker concentration $A = 1/2k_{-}/k =$ one-half the inhibition constant. This maximum in the curve of noise power vs. blocker concentration was experimentally observed by Van Driessche and Zeiske [29] and shown in their computer simulation.

Our three-state model also predicts such a maximum. To see how it depends on sodium concentration, Chen's [26] matrix formalism is useful. The variable whose power spectrum we want is the single-channel current. Written as a vector in (R_o, R_A, R_l)-space it is

$$X = i_o(1 \cdot R_o + 0 \cdot R_A + 1 \cdot R_l) \tag{23}$$

since the channel is open in the 'o' and 'l' states and closed in the 'A' state. Chen's α-matrix allows three variables. We can just call the two others zero for simplicity, writing

$$\alpha = i_o \begin{pmatrix} 1 & 0 & 1 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \tag{24}$$

Chen's Eqn. 28 for the low-frequency plateau of the power spectrum is *

$$G(0) = \frac{1}{\pi} \alpha' A'^{-1} \sigma' \tilde{\alpha}' \tag{25}$$

where

$$\alpha'_{\beta i} = \alpha_{\beta i} - \alpha_{\beta 0}$$

Again, the subscript zero refers to whichever of the linearly dependent variables is suppressed. For example, if we choose R_o and R_A as independent variables, then

$$\alpha' = i_o \begin{pmatrix} 0 & -1 \\ 0 & 0 \end{pmatrix} \tag{26}$$

The variance-covariance matrix of the three probabilities (Bernoulli formula) is

$$\sigma = \begin{pmatrix} R_o(1-R_o) & -R_oR_A & -R_oR_l \\ -R_oR_A & R_A(1-R_A) & -R_AR_l \\ -R_oR_l & -R_AR_l & R_l(1-R_l) \end{pmatrix}$$

$$= (1+a+s)^{-2} \begin{pmatrix} a+s & -a & -s \\ -a & a(1+s) & -as \\ -s & -as & (1+a)s \end{pmatrix} \tag{27}$$

The truncated 2 × 2 matrix is

$$\sigma' = (1+a+s)^{-2} \begin{pmatrix} a+s & -a \\ -a & a(1+s) \end{pmatrix} \tag{28}$$

Performing the indicated matrix multiplications, the surviving (oo) element of the power-spectrum matrix is

$$G(0) = \frac{1}{\pi} \frac{i_o^2}{k_-} \frac{a}{(1+a+s)^3} \left[(1+s)^2 + \xi as \right] \tag{29}$$

Suppose ξ small enough that the last term is negligible. Differentiating the expression $a/(1 - a + s)^3$ with respect to a to find the maximum, we see that the noise level peaks at $a = \frac{1}{2}(1 + s)$ (see Fig. 3 for graph). This predicted peak has been observed with triamterene [13]. The reason it did not show up in Frehland's [23] Fig. 4 is that those calculations kept $k_{op} = k_{cl}$ (his k_{op} is our k_-; his k_{cl} is our kA).

In interpreting data on the sodium-dependence of the noise power, it should be borne in mind that the open-channel current, i_o, is likely to be proportional to the apical sodium concentration. Looking at Eqn. 29, that puts an extra factor of s^2 implicitly in the numerator. For small values of the sodium concentration, the noise power will increase steeply with increasing s. Again it will show a maximum, because of that third-power term in the denominator. For higher sodium concentrations, adding sodium will suppress the noise.

Going to the four-state model brings in noise

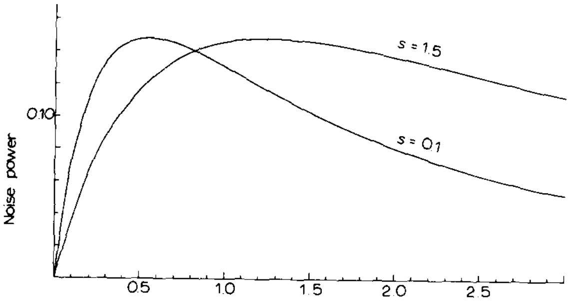

Fig. 3. Noise power (low-frequency plateau value) as a function of blocker concentration for two different sodium concentrations, according to Eqn. 29, with $i_o^2/\pi k_-$ set equal to unity: noise power $= a(1+s)^2/1 + a + s)^3$. Dimensionless units for a and s defined in Eqn. 7.

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* Chen's Eqn. 4 should have the factor 2 replaced by $1/2\pi$. All his subsequent expressions for $G(\omega)$ should therefore be multiplied by $1/4\pi$. But this error is cancelled out if angular frequency, ω, is replaced by frequency $f = \omega/2\pi$. The same error appears in Frehland and Stephan [17], Eqn. 3.6. Note the convention $\int_{-\infty}^{\infty} G(\omega) d\omega = \sigma_I^2$ (see, for example, Ref. 32). When using f instead of ω, the convention is to integrate over positive frequencies only.

due to sodium clogging. There is a third Lorentzian, with corner frequency so low as to be below the frequency range of measurements designed to study blocking noise, i.e., well below 1 Hz. Calculation of the plateau value according to Eqn. 25 involves a matrix product of four 3 × 3 matrices. To make it possible to appreciate the result we again resort to the device of letting the ratios

$$\xi = k_-/k_{-1},\ \eta = k_{-2}/k_- \text{ and } \zeta = k_2k_{-1}/k_1k_-$$

all be small ($\ll 1$), and discarding second-order terms.

Written as a vector in the space of the four states R_o, R_A, R_1 and R_2, the channel current is

$$X = i_o(1\cdot R_o + 0\cdot R_A + 1\cdot R_1 + 0\cdot R_2) \tag{30}$$

Choosing R_o, R_1 and R_2 as the linearly independent variables, the nonzero row of the $\mathfrak{a}'$-matrix is $i_o(1,1,0)$. The matrix product $\mathfrak{a}'\boldsymbol{B}\tilde{\mathfrak{a}}'$ again has only one surviving element (oo), consisting of the sum $B_{oo} + B_{o1} + B_{1o} + B_{11}$, where $\boldsymbol{B}$ is any matrix. Here B represents the product $A'^{-1}\boldsymbol{\sigma}'$. Setting $k_{-1} = 1$ for convenience, we have

$$A' = \begin{pmatrix} s+\xi+\xi a & -1+\xi & \xi \\ -s & 1+\xi\zeta s & -\xi\eta \\ 0 & -\xi\zeta s & \xi\eta \end{pmatrix} \tag{31}$$

Its determinant is $\xi^2\eta(1 + a + s + \beta s^2)$ (see Eqn. 32, below). To calculate the variance-covariance matrix $\boldsymbol{\sigma}$ we need the steady-state probabilities, R_i^s. A glance at the reaction scheme, Eqn. 13, gives

$$R_A^s/R_o^s = a,\ R_1^s/R_o^s = s,\ R_2^s/R_1^s = \beta s \tag{32}$$

with $\beta = k_2k_{-1}/k_1k_{-2}$. Note that β is a ratio of two inhibition constants, and may be greater than unity, unlike our other Greek-letter parameters. The normalization condition $R_o + R_A + R_1 + R_2 = 1$ yields

$$R_o^s = 1/(1+a+s+\beta s^2) \tag{33}$$

Accordingly, the variance matrix is

$$\sigma = (1+a+s+\beta s^2)^{-2} \times \begin{pmatrix} a+s+\beta s^2 & -s & -\beta s^2 \\ -s & s(1+a+\beta s^2) & -\beta s^3 \\ -\beta s^2 & -\beta s^3 & \beta s^2(1+a+s) \end{pmatrix} \tag{34}$$

Performing the inversion of the matrix of Eqn. 31 and multiplying it by the matrix of Eqn. 34 according to the prescription of Eqn. 25, keeping only the two largest terms yields for the low-frequency limit (the plateau) of the spectrum of current fluctuations

$$G(0) = \frac{1}{\pi}\frac{i_o^2(1+s)^2}{(1+a+s+\beta s^2)^3}\left[\frac{\beta s^2}{k_{-2}} + \frac{a}{k_-}\right] \tag{35}$$

The second term in the square bracket comes from the blocking, and is familiar from the three-state model Eqn. 29. The first term is the principal contribution of the sodium clogging. If, as we conjecture, the dissociation rate, k_{-2}, of the two-sodium-ion clog is very slow, this first term may well be the dominant contribution. At frequencies above the bottom corner ω_{clogging}, this clogging noise will fall off as the inverse square of the frequency, of course. Its signature will be a steep increase with sodium concentration at small enough values of s, indeed a fourth-power dependence if i_o is proportional to the sodium concentration. The blocking noise does not fall off with increasing frequency until frequency × 2π exceeds ω_{blocking}. There may be several octaves between the corners ω_{clogging} and ω_{blocking}, in which clogging noise ('sodium noise') falls off and blocking noise ('amiloride noise') does not.

For small enough blocker concentrations, the clogging noise will be greater than the blocking noise at all frequencies. As blocker concentration is increased from zero, the clogging noise power falls off. This is especially marked at low sodium concentrations, as a glance at that cubic term in the denominator of Eqn. 35 proves. That $(1 + \boldsymbol{a})^3$ in the denominator talks hard to suppress the clogging noise as $\boldsymbol{a}$ is increased. This suppression of the 'sodium noise' by rather small amounts of blocking agent has been observed (Hoshiko and Van Driessche, unpublished data), as has its analogue in potassium noise blocked by tetraethylamine [30]. It is a feature exhibited by any kinetic model in which the blocker competes with the transported ion species for the entrance site.

(III) Critique

The models we have treated are highly economical kinetic models, making no appeal to membrane structure. The 'states of the channel' refer only to the states of the transport sites. The channel has been assumed to be either 'open' or 'closed', ignoring the possibility of multi-conductance states. We have postulated a mean single-channel current, i_o, in the open channels without distinguishing whether the channel is in state 'o' or state '1', presumably taking some sort of average. We have not asked how i_o depends on voltage or on [Na]. One expects i_o to be roughly proportional to [Na] at low [Na], perhaps showing some saturation as the probability R_1 becomes non-negligible. Here is another possible mechanism of sodium self-inhibition to which we have closed our eyes by hiding it in the parameter i_o.

The economy of the models shows up also in the small number of parameters. There are the six rate constants, the single-channel current i_o, and the number of channels per unit area. To the extent that DC measurements at different sodium and blocker concentrations fit the models, such data can be used to evaluate the three equilibrium constants k_-/k, k_{-1}/k_1 and k_{-2}/k_2 via Eqn. 23 (steady-state values). In analyzing noise data, the fluctuation spectra are fitted to Lorentzian shapes. Each Lorentzian is characterized by two numbers, a corner frequency and a plateau value. By experiments at different sodium and blocker concentrations the various parameters can be highlighted in turn.

(IV) Overview

Our three-state model was devised to explain the sodium dependence of the corner frequency characterizing current fluctuations due to the blocking agents amiloride or triamterene. The fourth state was added in order to account for sodium self-inhibition and the low-frequency 'sodium noise' via a two-ion 'clogging' phenomenon. Our three-state model is a special case of our four-state model. Indeed, for sufficiently low sodium concentrations, few channels will be clogged. In addition to describing the concentration-dependence of the corner frequency, the models turn out to describe the concentration-dependence of noise power. They predict (1) the suppression of 'blocker noise' power by high sodium concentration, (2) the suppression of blocker noise power by high blocker concentration, and (3) the suppression of 'clogging noise' power by increased blocker concentration. These effects arise naturally from the sodium interdiction of amiloride binding and vice versa. The models are in qualitative agreement with data available thus far. It remains to be seen if more quantitative data will bear out their predictions in detail.

Acknowledgements

We would like to acknowledge helpful discussions with Dr. Hans-Helmut Kohler, Professor Bernd Lindemann, Professor Wolfram Nagel, Dr. Roger Rick, and Dr. Willy Van Driessche. The work has been supported in part by the Deutsche Forschungsgemeinschaft (E.F.) and by the National Institutes of Health AM05865 (T.H. and S.M.).

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