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The Properties of Ionic Channels Measured by Noise Analysis in Thin Lipid Membranes

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Summary. Noise analysis was performed on the membrane currents produced by the ion channel former gramicidin A in black lipid bilayer membranes. The average channel lifetime and the unit channel conductance can be determined from the autocorrelation function. The values agree with the independently obtained data from measurements of single channels. The dependence of this function on the channel density reveals information on the process of channel formation. The kinetic information is the same as that obtained by voltage clamp measurements.

Key words: Noise Analysis — Ionic Channel Properties — Gramicidin A — Black Lipid Membranes — Autocorrelation.

Noise measurements on the ion currents across nerve membranes have been introduced into neurophysiology recently (Verveen and Derksen, 1968). They led to an estimate for the magnitude of the conductance increments produced by the opening of individual ionic channels (Katz and Miledi, 1972; Anderson and Stevens, 1973; Siebenga *et al.*, 1973) and provided information on the kinetics and voltage dependence of acetylcholine gates (Katz and Miledi, 1972; Anderson and Stevens, 1973). There is also the expectation that noise analysis will allow a decision between various classes of models for the voltage dependent conductance changes in nerve membranes (Stevens, 1972; Fishman, 1973).

Theories suggesting noise analysis as an analytical tool were established many years ago (Groot and Mazur, 1962). Measurements on well characterized physico-chemical systems, proving the applicability of the concept, have been scarce. In fact, successful applications of noise analysis in physiology still outnumber applications to problems of chemical reaction kinetics. Therefore it seemed worthwile to take an effort in testing the method on a simple system.

Discrete conductance steps representing the opening and closing of individual ionic channels are produced in black lipid membranes by a number of polypeptides, the antibiotic gramicidin A being the best characterized ion channel former (Haydon and Hladky, 1972). The random superposition of many single events leads to higher membrane

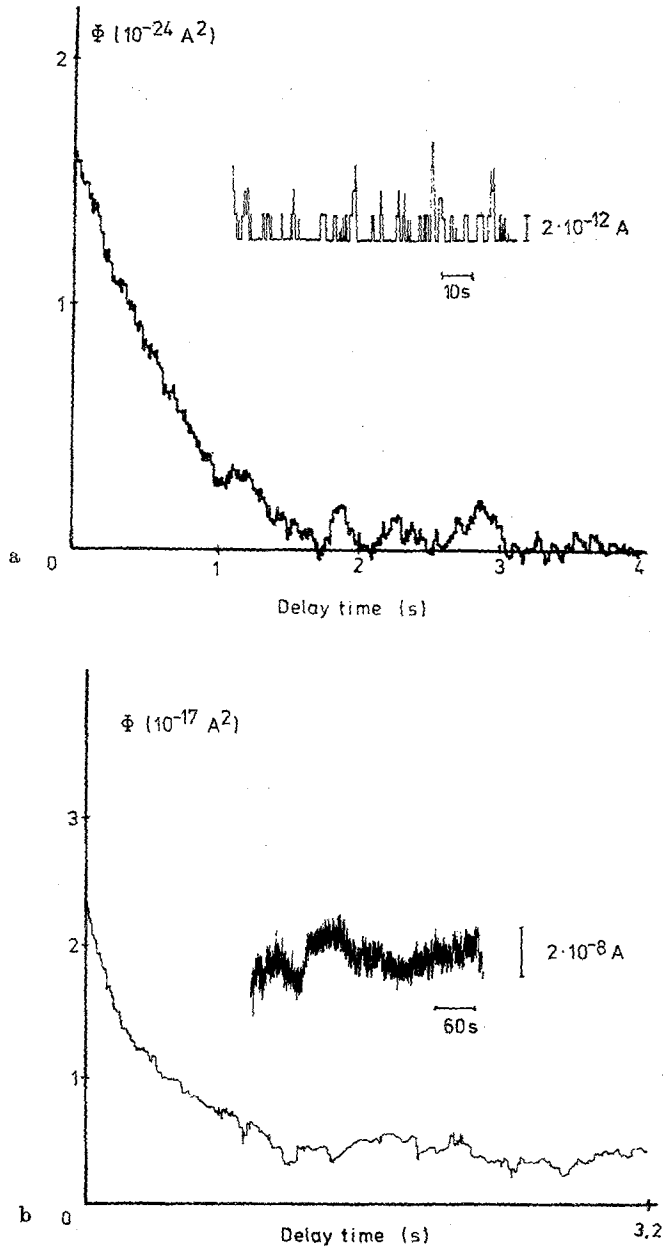

Fig. 1. (a) Autocorrelation function obtained at the unit channel conductance level. Single ion channels were produced by small amounts of gramicidin A in black lipid membranes formed from solutions of dioleoyl-L-α-lecithin in *n*-decance at 1 M KCl. Insert: Direct recording of a few current fluctuations. The autocorrelation function

conductance values and allows the resulting current noise to be related to the properties of the single channel, i.e. the unit channel conductance and the mean channel lifetime. It was shown that even in single channel experiments (see insert of Fig. 1 a) the conductance steps are not produced by one and the same channel opening and closing (Hladky and Haydon, 1972). The current is rather the sum of the effects of independently ocurroing channels at different locations.

Measurements and Analysis of the Experimental Results

Black lipid membranes were formed from solutions of dioleoyl-L-α-lecithin in n-decane by conventional techniques over a small hole in a polyethylene vessel submerged in an aqueous salt solution (1 M KCl). A constant voltage of 100 mV was applied. The membrane current was measured after the addition of small amounts of gramicidin A from an ethanolic stock solution.

Current noise was analyzed with a "Fabritek" Model 1074 Instrument Computer (Nicolet Instruments Corporation) equipped with a Model SD-75A auto/cross correlation digitizer. The autocorrelation function (Dern and Walsh, 1963) calculated by this instrument represents the average time course, which a spontaneous microscopic deviation from equilibrium (a statistical fluctuation) takes in returning to that state. For simple systems it is an exponential with the same time constant τ as obtained from a voltage clamp experiment (Chen and Hill, 1973). Moreover, at low conductivity levels (small channel densities) this time constant equals the average lifetime τ_0 of the conducting channel.

The Average Channel Lifetime Can be Measured by the Autocorrelation Function

The insert of Fig. 1 a shows an example of conductance changes due to the opening and closing of single channels. This result is obtained, if very small amounts of gramicidin A are introduced into the membrane. The main part of Fig. 1 a presents the autocorrelation function from the same experiment. The time constant $\tau = 0.78 \pm 0.05$ s is in excellent agreement with the average channel lifetime determined by manual measurement of the same 300 recorded single channels ($\tau_0 = 0.76 \pm 0.03$ s). At higher conductivities the time constant remains unchanged (within 10%)

was obtained from approx. 300 single events. Membrane area approx. $2 \cdot 10^{-3}$ cm^2. (b) Autocorrelation function obtained at a high conductivity level (approx. $1.5 \cdot 10^{10}$ channels per cm^2). Insert: Recording of the current noise signal (ac-component only) from which the autocorrelation function was calculated. Membrane area approx. $2 \cdot 10^{-3}$ cm^2

up to a conductivity level, where the current represents the superposition of $3 \cdot 10^4$ channels (channel density $2 \cdot 10^7$ cm^{-2}).

The Unit Conductance-Step Can be Measured by the Autocorrelation Function

From the definition of the autocorrelation function (Dern and Walsh, 1963) it follows that its amplitude is equal to the variance of the measured quantity[1]. By σ_λ^2 we denote the variance of the membrane conductivity, $\bar{\lambda}$. σ_λ^2 was obtained from σ_I^2 by dividing this value by the membrane area and the square of the applied potential[2].

The quantity $\sigma_\lambda^2/\bar{\lambda}$ equals the unit conductance step Λ, provided the single events are independent (Rice, 1944). For the investigated model system $\sigma_\lambda^2/\bar{\lambda}$ yielded the correct values for Λ (within $10\,^0/_0$) up to $3 \cdot 10^4$ superimposed single channels (channel density $2 \cdot 10^7$ cm^{-2}).

The Concentration Dependence of the Autocorrelation Functions Carries Information on the Process of Channel Formation

At higher channel densities ($> 10^6$ cm^{-2}), which may be obtained by adding more gramicidin A, the values for both τ and $\sigma_\lambda^2/\bar{\lambda}$ decrease in a characteristic manner. This behaviour expresses the fact that the individual channels are no longer independent. It provides information on the process which leads to the formation of a single channel. Fig. 1b shows an example of the autocorrelation function obtained at a conductivity equivalent to a channel density of $1.5 \cdot 10^{10}$ cm^{-2}. The insert shows a recording of the actual noise signal. In Fig. 2a the values of $\frac{1}{\tau}$ (closed circles) have been plotted against $\sqrt{\bar{\lambda}}$. The solid straight line is the theoretical prediction (Bamberg and Läuger, 1973) based on the assumption that the conducting channel is formed by a dimerization reaction between two gramicidin A molecules. This reaction scheme was proposed by Urry (1971) and Urry *et al.* (1971) and adopted by Bamberg and Läuger (1973) in their voltage clamp study on the same system. Slope and inter-

1 The amplitude of the autocorrelation function is the difference between its initial value $\Phi(0)$ and the value $\Phi(\infty)$ at infinite time. $\Phi(0)$ is the mean squared value and $\Phi(\infty)$ the square of the mean value of the membrane current. $\Phi(0) - \Phi(\infty) = \overline{I^2} - (\bar{I})^2 = \sigma_I^2$, where I denotes the membrane current. The signal was passed through a high pass filter to remove the d.c.-component. Therefore $\Phi(\infty) = 0$.

2 The validity of this normalization becomes evident if the operation is looked upon as the superposition of the contributions of many independent membranes so that the individual areas add up to 1 cm^2. (The variance of the sum of independent processes is equal to the sum of the variances.) Thus, σ_λ^2 has the dimension Ω^{-2} cm^{-2}. Its value is equal to the variance of conductance that would be obtained for a membrane of 1 cm^2 area under otherwise equal conditions.

cept were chosen to obtain a least squares fit. The intercept (equivalent to an extrapolation to $\bar{\lambda} = 0$) yields the dissociation rate constant $k_d = \frac{1}{\tau_0}$, whereas the association rate constant k_r can be calculated from the slope. Fig. 2b shows the result of the amplitude analysis at higher conductivities. The quantity $\frac{\bar{\lambda}}{\sigma_\lambda^2}$ is plotted versus $\sqrt{\bar{\lambda}}$. Again the solid line represents the theoretical result predicted from the known value of Λ (giving the intercept $1/\Lambda$) and from the equilibrium constant $K = \frac{k_r}{k_d}$ (calculated from Fig. 2a), which is required for calculating the slope.

Comparison of Methods (Voltage Clamp, Autocorrelation Function, Power Spectrum)

In another paper we have demonstrated in detail that relaxation methods (of which the voltage clamp technique is a particular example) and the statistical analysis of concentration fluctuations can yield equivalent results (Zingsheim and Neher, 1974). This is not only an empirical finding, but also to be expected from the fluctuation dissipation theorem of irreversible thermodynamics (Groot and Mazur, 1962).

We would also like to point out that statistical analysis of noise signals can be performed by measuring either the autocorrelation function or the power spectrum. Both methods provide the same information. The power spectrum is the Fourier transform of the autocorrelation function. In our experience, however, the use of autocorrelation is more convenient for the measurement of aperiodic processes (noise) in membranes:

a) the presentation of the kinetic information in the time domain is more familiar to workers acquainted with the voltage clamp technique or chemical relaxation methods,

b) experimental artifacts, which in most cases show up as slow fluctuations, are represented by a few widely scattered points in the power spectrum, whereas they may be well separated off by the selection of a proper base line of the autocorrelation function,

c) the amplitude information can be easily read off the autocorrelation function, whereas otherwise it would be necessary to determine the area under the power spectrum.

Still, we regard these technical questions as only of secondary importance. They may be largely a matter of personal preference.

We have presented here an example where noise analysis can provide the parameters of the single channel (unit conductance step, mean channel life time), if the measurements are performed at low membrane concentrations of the reactants. We have been able to compare the results with values obtained from direct measurements of the same quantities.

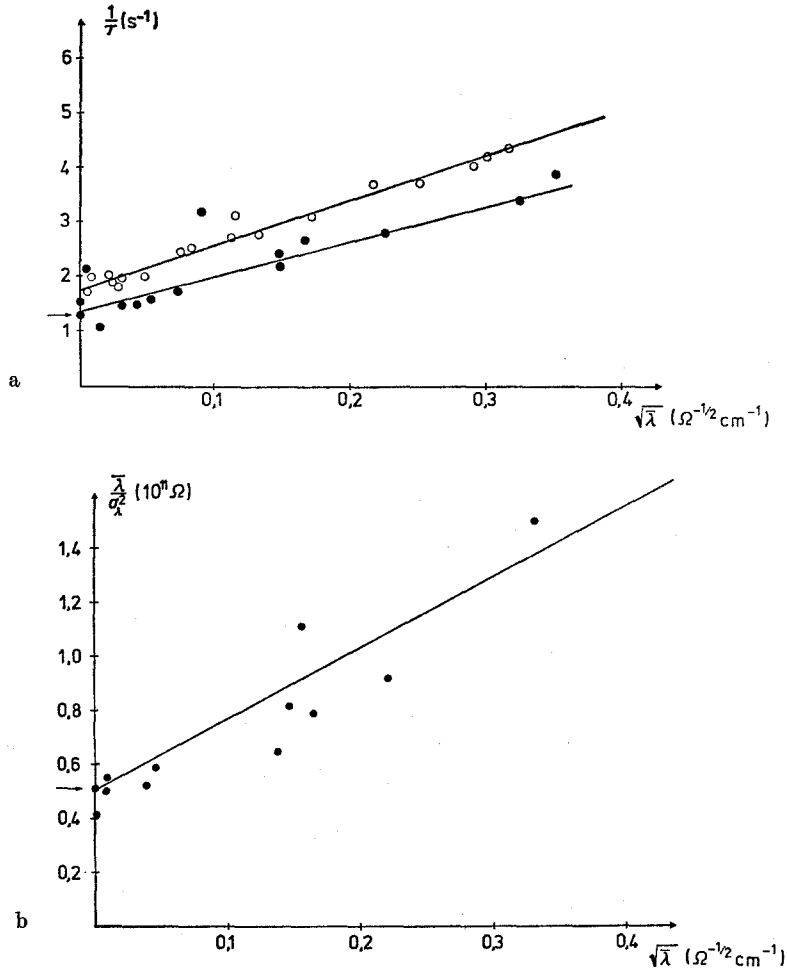

Fig. 2. (a) Dependence of the reciprocal time constants on membrane conductivity. $\frac{1}{\tau}$ is plotted versus $\sqrt{\bar{\lambda}}$ (see text). • correlation measurements ($k_r = 2.5 \cdot 10^{13}$ M^{-1} cm^2 s^{-1}, $k_d = 1.4$ s^{-1}); ○ control measurements by voltage clamp ($k_r = 2.9 \cdot 10^{13}$ M^{-1} cm^2 s^{-1}, $k_d = 1.8$ s^{-1}). Dioleoyl-L-α-lecithin membranes at 1 M KCl. The y-axis intercept yields the reciprocal value of the mean unit channel lifetime. The dominating experimental artifacts are different for correlation and voltage clamp measurements (see Zingsheim and Neher, 1974). Therefore the discrepancy between the results obtained by the different methods cannot be considered significant. (b) Dependence of $\frac{\bar{\lambda}}{\sigma_\lambda^2}$ on $\sqrt{\bar{\lambda}}$ (see text). Dioleoyl-L-α-lecithin membranes at 1 M KCl. The y-axis intercept yields the reciprocal value of the unit channel conductance. The arrows indicate the values obtained from single channel measurements

Even more, we want to emphasize that further information may be gained if the concentration dependence of the measured quantities is also taken into account. This latter point has not yet been generally appreciated in applications of noise analysis to problems of membrane physiology.

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