Fluctuation-Driven Directional Flow in Biochemical Cycle: Further Study of Electric Activation of Na,K Pumps

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ABSTRACT Directional flow of information and energies is characteristic of many types of biochemical reactions, for instance, ion transport, energy coupling during ATP synthesis, and muscle contraction. Can a fluctuating force field, or a noise, induce such a directional flux? Previous work has shown that Na,K-ATPase of human erythrocyte can absorb free energy from an externally applied random-telegraph-noise (RTN) electric field to pump Rb^+ up its concentration gradient. However, the RTN field used in these experiments was constant in amplitude and would not mimic fluctuating electric fields of a cell membrane. Here we show that electric fields which fluctuate both in life time and in amplitude, and thus, better mimicking the transmembrane electric fields of a cell, can also induce Rb^+ pumping by Na,K-ATPase. A Gaussian-RTN-electric field, or a field with amplitude fluctuating according to the Gaussian distribution, with varied standard deviation (σ), induced active pumping of Rb^+ in human erythrocyte, which was completely inhibited by ouabain. Increased values for σ led to a nonmonotonic reduction in pumping efficiency. A general formula for calculating the ion transport in a biochemical cycle induced by fluctuating electric field has been derived and applied to a simple four-state electroconformational coupling (ECC) model. It was found that the calculated efficiency in the energy coupling decreased with increasing σ value, and this effect was relatively small and monotonic, whereas experimental data were more complex: monotonic under certain sets of conditions but nonmonotonic under different sets. The agreement in general features but disagreement in some fine features suggest that there are other properties of the electric activation process for Na,K-ATPase that cannot be adequately described by the simple ECC model, and further refinement of the ECC model is required.

INTRODUCTION

A cell can transduce signal or energy that is contained in an oscillatory or a fluctuating force field (Astumian et al., 1987; Astumian and Bier, 1994; Chen, 1987; Horn, 1993; Tsong and Astumian, 1986; Tsong, 1990). This ability of a cell implies that mechanisms exist for molecules of cells to receive, process, and generate oscillatory or fluctuating force fields (Tsong, 1989, 1991). Theories based on electroconformational coupling (ECC) have established that an enzyme undergoing a cyclic reaction is a microscopic device that can absorb energy from a periodic or fluctuating driving force and couple the absorbed energy to fuel a free energy-consuming chemical reaction, for instance, ATP synthesis, pumping of an ion up its concentration gradient, or muscle contraction (Tsong and Astumian, 1986; Astumian et al., 1987; Chen, 1987; Astumian and Bier, 1994). In essence, such an enzyme is an internal motor or resonator that can interact with an external or internal force field. There are some restrictions that are necessary for such a energy-transducing system to work. The most fundamental ones are that the enzyme must have at least one degree of freedom restricted and it must be able to interact with the applied field. A free-floating molecule can be an effective energy transducer only if the rotational relaxation time of the molecule is much longer than the mean periodicity of the force field. Absorption of radiation energy by a chromophore to become an excited molecule is an example.

Previous experiments with Na,K-ATPase have revealed several interesting properties of the electroactivation process (Serpersu and Tsong, 1983, 1984; Liu et al., 1990; Xie et al., 1994). Only those ouabain-sensitive ion pumping modes of the enzyme are found to be subjected to electric field action. Ion leaks or passive transports are not affected by electric stimulation. These results imply that the electric activation of the Na and the K pumps follows mechanisms similar to that of the ATP-dependent activation. Furthermore, the field-induced ion pumps have characteristic windows with respect to electric field frequency and amplitude. The optimal frequency for the Na^+-pumping mode is 1.0 MHz, and that for the K^+-pumping mode is 1.0 kHz. The optimal amplitudes for the two pumping modes are identical, i.e., 20 V/cm. These values are the same whether a regularly oscillating field or a constant-amplitude RTN field was used (Xie et al., 1994). This last point is particularly intriguing, because it has been shown recently that charged Brownian particles are able to undergo directional movement, even against a retarding force, when they are exposed to a periodic electric potential that is asymmetrical within a period, and is switched on and off regularly or randomly (Astumian et al., 1987; Chen, 1987; Astumian and Bier, 1994; Rousselet et al., 1994; Faucheux et al., 1995; Zhou

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Received for publication 3 June 1996 and in final form 28 February 1997.

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0006-3495/97/06/2496/07 $2.00

and Chen, 1996). In other words, the energy contained in a fluctuating electric field can be absorbed by a charged Brownian particle to do mechanical work.

One interesting theoretical problem related to this type of free energy transduction is how to calculate or evaluate the fluctuation-induced flux in a biochemical cycle of arbitrary complexity. Using stochastic argument, Chen (1987) has shown that the flux can be obtained from an expanded diagram if the fluctuation is of the RTN type, in which only the "dwelling time" of the signal is fluctuating, but the amplitude is constant. In this paper, both the electric activation experiment and theoretical analysis will be extended to cases in which the amplitude of the RTN field is also fluctuating. A formalism for the general iteration procedure will be derived, and simulation based on a simple four-state ECC model will be compared with experimental results.

MATERIALS AND METHOD

Two IBM-compatible PCs (Dell Computer System 310, CPU80386, 20 MHz) were used to drive a functional generator to produce a train of RTN field of fluctuating amplitudes. One PC generated an amplitude value, x, according to a Gaussian distribution function,

$$G(x) = \frac{1}{\sigma\sqrt{\pi}}\exp\left(\frac{(x-\mu)^2}{2\sigma^2}\right)$$

where μ and σ are, respectively, the mean and the standard deviation of the distribution. This PC controlled the voltage output of a Wavetek Model 166, 50-MHz pulse/function generator through a digital-to-analog converter. The second PC generated a triggering signal to control the pulse width of the functional generator. The duration of each pulse, t, was exponentially distributed: $t = -\tau^* \ln(R)$, where τ^* is the mean lifetime used for an experiment and R is a random number between 0.01 and 0.99. The mean frequency values, $f^* = 1/(2\tau^*)$, in the range of 100 Hz to 50 kHz were used in the experiment. This setup and its operation procedure have been described elsewhere (Xie et al., 1994). As is clear from this description, there were some limitations for the experimental setup. The most obvious one is the cutoff point for the distribution at $x = 2\sigma$. This was necessary because of the limited voltage capacity of the functional generator. This limitation in instrumentation is judged not to be serious, because the amplitude window for the electric activation is relatively narrow (Liu et al., 1990; Xie et al., 1994). With this setup, three types of waveforms were generated and tested on Na,K-ATPase. They are RTN field of constant amplitude (Fig. 1 *A*), squared wave of constant lifetime with Gaussian distribution of amplitude (Fig. 1 *B*), and RTN field with Gaussian distribution (Fig. 1 *C*).

Ouabain-sensitive Rb^+ pumping activity was assayed as described previously (Liu et al., 1990; Xie et al., 1994). Human erythrocytes were prepared from freshly drawn blood and incubated in an isotonic medium containing RbCl to preload Rb^+ into the cytoplasm. The cytoplasmic concentrations of the erythrocyte samples used for the electric activation experiment were 5 mM for Na^+, 16 mM for Rb^+, and 105 mM for K^+. The external medium contained 10 mM Rb^+, 0.5 mM $MgCl_2$, 10 mM Tris-HCl at pH 7.4, 5–140 mM NaCl, and an appropriate amount of sucrose to adjust the solution to isotonicity. A small amount of $^{86}Rb^+$ was loaded into the cytoplasm when the efflux of Rb^+ was to be measured. Conversely, when influx of Rb^+ was to be measured, the tracer was added in the external medium. As was shown previously, an electric field at the level used in the activation experiment does not cause passive leaks in Rb^+ efflux and influx. For a given set of experimental conditions, Rb^+ influx was measured for four samples: the control, or nonstimulated sample, without ouabain (NS); nonstimulated sample with 0.2 mM ouabain (ONS); stimulated sample without ouabain (S); and stimulated sample with 0.2 mM ouabain (OS). Electric stimulation was done for 30 min at 3 ± 1°C. Rb^+ flux was expressed in attomoles (10^{-18} mole) per red cell per h, following the method of our previous publications. The quantity (NS − ONS) measures ATP-dependent Rb^+ pumping, which at 3°C was negligibly

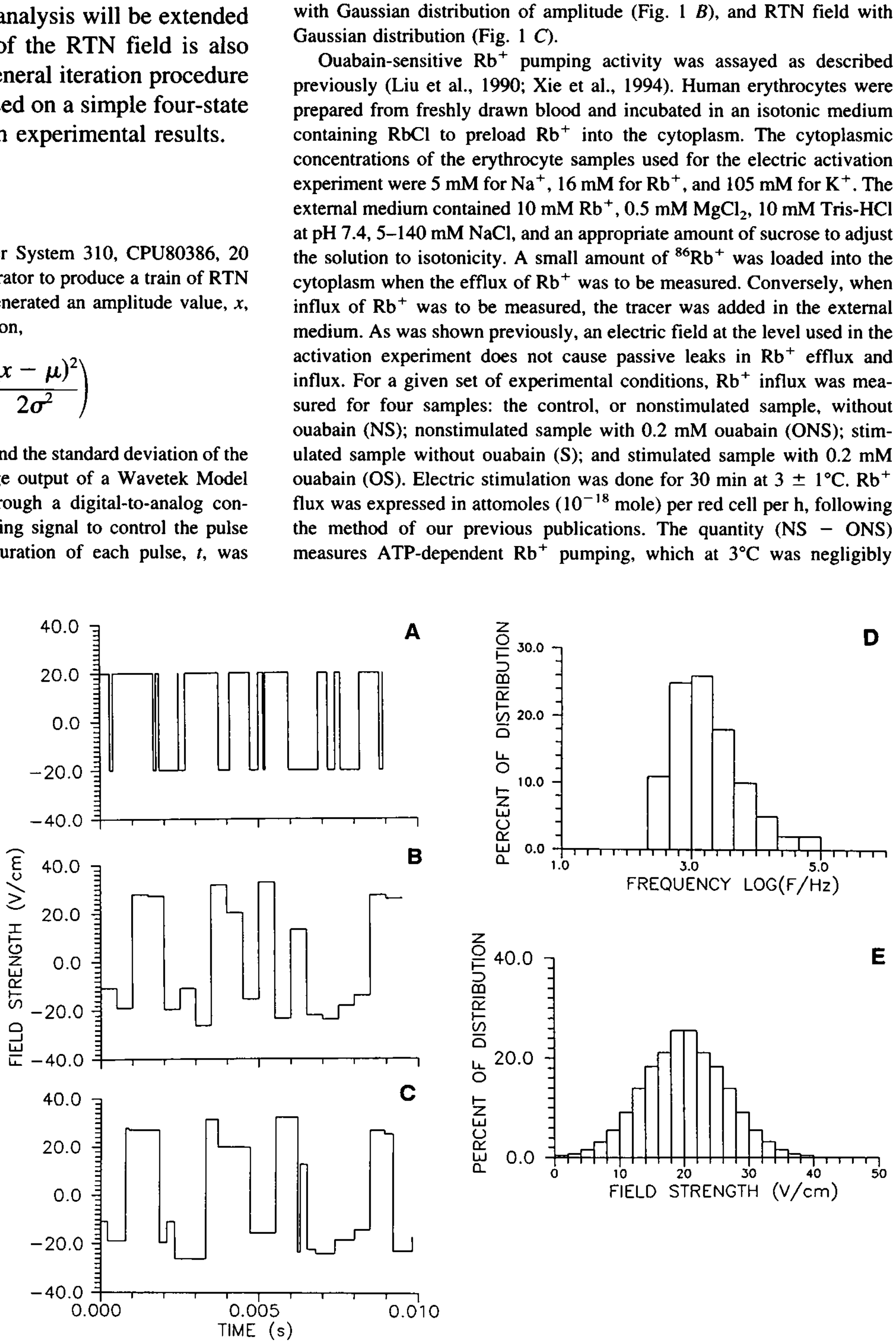

FIGURE 1 Typical waveforms of electric fields used in the experiment. (*A*) A RTN with a constant amplitude (±20 V/cm) and a fluctuating lifetime. (*B*) A waveform with a constant lifetime (1 kHz) and a fluctuating Gaussian-distributed amplitude. (*C*) A RTN with fluctuating amplitude and lifetime. (*D*) A histogram of frequency distribution for waveforms shown in *A* and *C*. (*E*) A histogram of a Gaussian amplitude distribution, with σ = 10 V/cm, for the waveforms shown in *B* and *C*.

small compared to the quantity (S − OS), which measures the net electric field-stimulated pumping activity.

RESULTS

Fluctuating electric-field-induced Rb^+ efflux

Figs. 2 and 3 show the effect of dispersion in amplitudes of a RTN field on the net Rb^+ influx in red blood cells. In Fig. 2, the net RTN field-stimulated, ouabain-sensitive Rb^+ influx is plotted as a function of the standard deviation (σ) of the amplitudes of the Gaussian distributed electric field at different values of the mean amplitude. The mean frequency of the RTN is fixed at 1.0 kHz. As shown in the figure, an increase in σ generally leads to a decrease in Rb^+ influx. However, the curves for the mean amplitudes of 15 V/cm and 10 V/cm show nonmonotonic dependence on σ. As will be shown later, the nonmonotonic characteristics of the plots are not reproduced by the model analysis based on a simple four-state ECC transporter model. Fig. 3 gives the results of an experiment in which the mean amplitude of the Gaussian distribution was fixed at 20 V/cm, and σ was varied, along with the mean frequency of the RTN field. The frequency optimum remained unchanged, at 1 kHz, but the Rb^+ influx decreased with increasing σ.

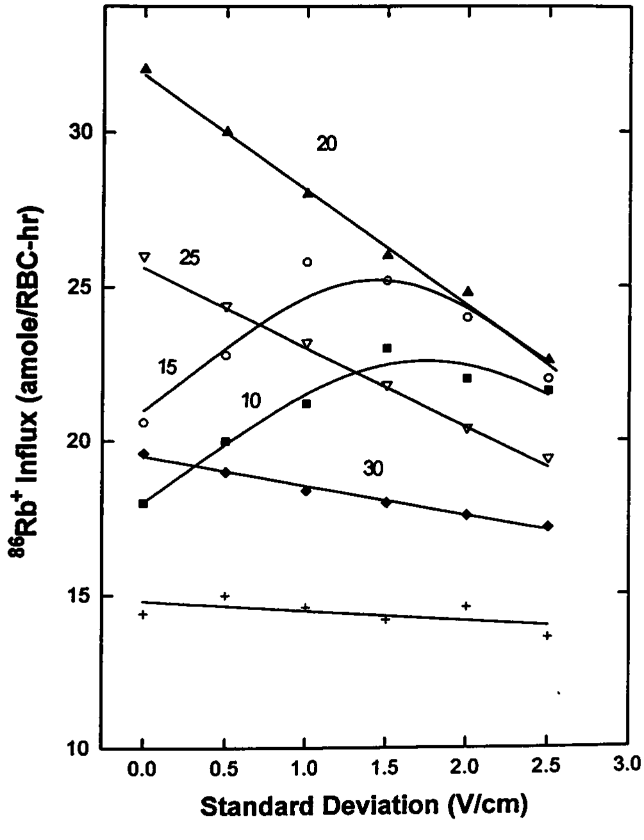

FIGURE 2 Electric field-induced, ouabain-sensitive Rb^+ uptake by human erythrocyte. Gaussian-RTN fields of different amplitudes (V/cm) at 1 kHz mean frequency (waveforms as in Fig. 1 C) were used. The influxes were measured and plotted as a function of the standard deviation (σ) of the amplitude distribution. The line with crosses represents the control (without electric stimulation).

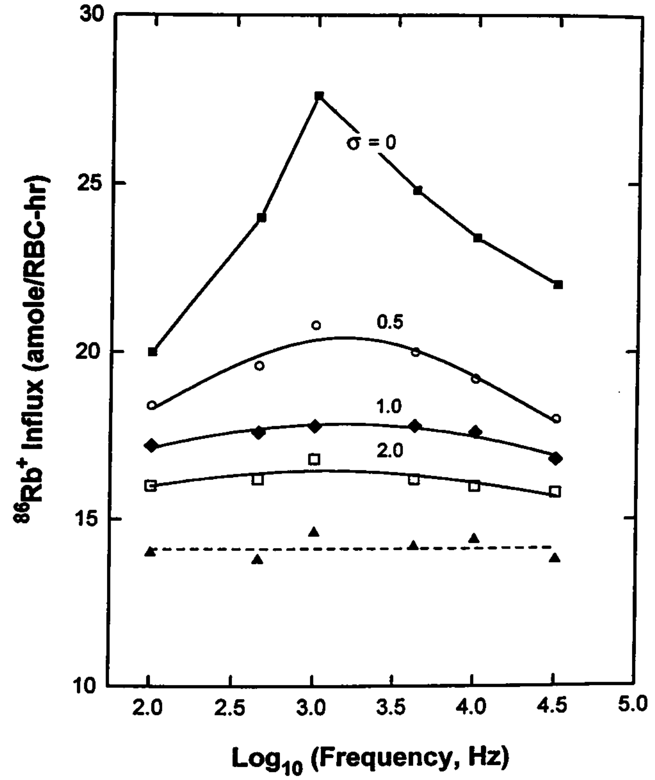

FIGURE 3 Gaussian-RTN-field-induced Rb^+ influx at a mean amplitude of 20 V/cm as a function of the frequency of the fluctuation at different σ values. The line with filled triangles denotes the control.

Theory and mathematical formulation

In a RTN, the random variable (a signal or a driving force) is fluctuating between positive and negative phases, forming a train of square pulses with exponentially distributed times of duration. If the value (amplitude) of the fluctuating variable in both the positive and the negative phases is fixed at a single value, the fluctuation is called a one-state RTN (or a dichotomous noise). If the amplitude can take two discrete values in each phase, the fluctuation is called a two-state RTN. In a Gaussian RTN, the amplitude in each phase is continuous and Gaussian distributed. Fig. 4 shows the kinetic schemes and the time histograms of these three types of RTN fields. The procedure to evaluate the steady-state flux of a biochemical cycle (or a multicycle system) under the influence of an externally applied one-state RTN has been discussed by Chen (1987) in terms of a diagram method. In this section, we extend the same method to the case in which the number of discrete amplitudes in each phase is two, and then present a numerical iteration procedure that is applicable to any kind of amplitude distribution, including the case in which the amplitude is not discrete but continuous.

Consider the kinetic scheme of the two-state RTN shown in Fig. 4 *B*, where the potential *V* is fluctuating between a positive and a negative phase with amplitudes Δ_1 and Δ_2. γ in the figure represents the transition probability between

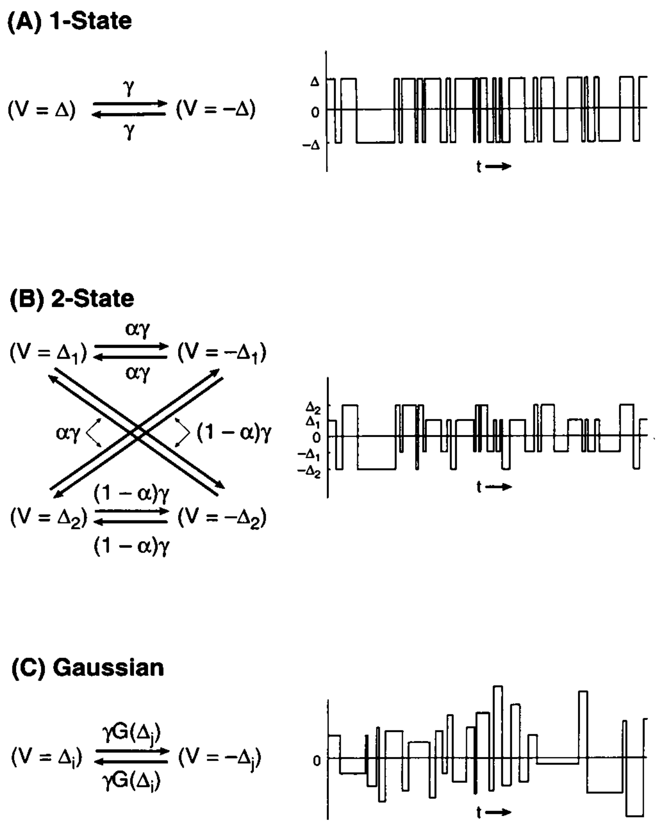

FIGURE 4 Kinetic schemes and histograms of some RTN signals. The signal V is fluctuating between positive and negative values with a mean frequency of $\gamma/2$. (*A*) The one-state RTN with one amplitude. (*B*) The two-state RTN, with amplitude being distributed between Δ_1 and Δ_2 with probability densities of α and $1 - \alpha$, respectively. The histogram on the right is generated with $\alpha = \frac{1}{2}$. (*C*) The RTN with Gaussian-distributed amplitudes.

the two phases, and α denotes the probability of V having the amplitude Δ_1 ($1 - \alpha$ is that for Δ_2). We want to know how to calculate the cycle flux of the four-state cyclic ECC transport model shown in Figure 3 of Xie et al. (1994) under the influence of this fluctuating V. Let $p_i^+(\Delta_1)$ be the probability of the system being in state i ($i = 1, 2, 3, 4$) at $V = +\Delta_1$ (the positive phase), etc., then following the same stochastic treatment of Chen (1987), we obtain

$$\frac{dp_i^+(\Delta_1)}{dt} = -\sum_{j\neq i} k_{ij}^+(\Delta_1)p_i^+(\Delta_1) + \sum_{j\neq i} k_{ji}^+(\Delta_1)p_j^+(\Delta_1) - \gamma p_i^+(\Delta_1) + \alpha\gamma[p_i^-(\Delta_1) + p_i^-(\Delta_2)], \tag{1}$$

$$\frac{dp_i^+(\Delta_2)}{dt} = -\sum_{j\neq i} k_{ij}^+(\Delta_2)p_i^+(\Delta_2) + \sum_{j\neq i} k_{ji}^+(\Delta_2)p_j^+(\Delta_2) - \gamma p_i^+(\Delta_2) + (1 - \alpha)\gamma[p_i^-(\Delta_1) + p_i^-(\Delta_2)], \quad i = 1, 2, 3, 4 \tag{2}$$

where $k_{ij}^+(\Delta_1)$ is the transition rate constant from state i to state j when the field is positive and the amplitude is at Δ_1. Similar equations can be obtained for the negative phases by exchanging the + and − superscripts in Eqs. 1 and 2. The first two terms in Eqs. 1 and 2 represent the rate of change caused by the chemical transitions of the cycle, and the last two terms represent the change caused by the fluctuation of the parameter V. Summing Eqs. 1 and 2 over the index i at steady state ($dp_i^+(\cdot)/dt = 0$), we obtain

$$\sum_i[p_i^+(\Delta_1) + p_i^+(\Delta_2)] = \sum_i[p_i^-(\Delta_1) + p_i^-(\Delta_2)] = \tfrac{1}{2} \tag{3}$$

This equation means that the sum of all biochemical states of the cycle in the positive phase is equal to that in the negative phase of V. This is not surprising, because V spends equal time on the two phases (so that $\langle V\rangle = 0$).

Setting the left-hand sides of Eqs. 1 and 2 to zero (at steady state), we obtain

$$\mathbf{M}^+(\Delta_1)\cdot\mathbf{P}^+(\Delta_1) = \alpha\gamma[\mathbf{P}^-(\Delta_1) + \mathbf{P}^-(\Delta_2)] \tag{4}$$

$$\mathbf{M}^+(\Delta_2)\cdot\mathbf{P}^+(\Delta_2) = (1 - \alpha)\gamma[\mathbf{P}^-(\Delta_1) + \mathbf{P}^-(\Delta_2)] \tag{5}$$

$$\mathbf{M}^-(\Delta_1)\cdot\mathbf{P}^-(\Delta_1) = \alpha\gamma[\mathbf{P}^+(\Delta_1) + \mathbf{P}^+(\Delta_2)] \tag{6}$$

$$\mathbf{M}^-(\Delta_2)\cdot\mathbf{P}^-(\Delta_2) = (1 - \alpha)\gamma[\mathbf{P}^+(\Delta_1) + \mathbf{P}^+(\Delta_2)] \tag{7}$$

where $\mathbf{M}$ is a 4 × 4 matrix:

$$\mathbf{M}^+(\Delta) = \begin{bmatrix} \sum_{j\neq i} k_{1j}^+(\Delta) + \gamma & -k_{21}(\Delta) & 0 & -k_{14}(\Delta) \\ -k_{12}(\Delta) & \sum_{j\neq 2} k_{2i}^+(\Delta) + \gamma & -k_{32}(\Delta) & 0 \\ 0 & -k_{23}(\Delta) & \sum_{j\neq 3} k_{3j}^+(\Delta) + \gamma & -k_{43}(\Delta) \\ -k_{14}(\Delta) & 0 & -k_{34}(\Delta) & \sum_{j\neq 4} \mathrm{k}_{4j}^+(\Delta) + \gamma \end{bmatrix} \tag{8}$$

and the $\mathbf{P}$'s are column vectors:

$$\mathbf{P}^+(\Delta_1) = \mathrm{col}[p_1^+(\Delta_1), p_2^+(\Delta_1), p_3^+(\Delta_1), p_3^+(\Delta_1)] \tag{9}$$

Equations 4–7 are the basic equations for evaluating the probabilities by iteration.

If we assign some values for the negative p's on the right-hand sides of Eqs. 4 and 5, the probabilities in the positive phase can be obtained from these two equations by matrix inversion. Note that the sum of the input p's must be equal to ½ (see Eq. 3). As a result, the sum of the resultant p's in the positive phase will also be equal to ½. By substituting the newly calculated positive phase p's into the right-hand sides of Eqs. 6 and 7, the state probabilities in the negative phase can also be evaluated by the matrix inversion. The calculation is repeated until the difference between the input and the output probabilities is within a certain error limit. This iteration procedure can be extended easily to Gaussian RTN or other types of RTN, as described below.

When the amplitude of the RTN is Gaussian distributed (or any other type of distribution), Eqs. 4 and 5 can be represented by a single equation as

$$\mathbf{M}^{+}(\Delta)\cdot\mathbf{P}^{+}(\Delta) = \gamma G(\Delta)\int_{-\infty}^{\infty}\mathbf{P}^{-}(\Delta)\,d\Delta \tag{10}$$

where $\mathbf{M}^{+}(\Delta)$ is given by Eq. 8 and $G(\Delta)$ is a Gaussian function of Δ. Similarly, Eqs. 6 and 7 can be represented by

$$\mathbf{M}^{-}(\Delta)\cdot\mathbf{P}^{-}(\Delta) = \gamma G(\Delta)\int_{-\infty}^{\infty}\mathbf{P}^{+}(\Delta)\,d\Delta \tag{11}$$

With a given $\mathbf{P}^{-}(\Delta)$, Eq. 10 can be used to obtain $\mathbf{P}^{+}(\Delta)$. This $\mathbf{P}^{+}(\Delta)$ in turn is used to evaluate $\mathbf{P}^{-}(\Delta)$ from Eq. 11, forming one round of iteration. After the steady-state probabilities in the positive and the negative phases are obtained by iteration, the flux (J) of the ligand transport is calculated as

$$\begin{aligned} J = {} & \int_{-\infty}^{\infty} k_{ij}^{+}(\Delta)p_i^{+}(\Delta)\,d\Delta - \int_{-\infty}^{\infty} k_{ji}^{+}(\Delta)p_j^{+}(\Delta)\,d\Delta \\ & + \int_{-\infty}^{\infty} k_{ij}^{-}(\Delta)p_i^{-}(\Delta)\,d\Delta - \int_{-\infty}^{\infty} k_{ji}^{-}(\Delta)p_j^{-}(\Delta)\,d\Delta \end{aligned} \tag{12}$$

where states i and j are two neighboring states in the cycle.

Model simulations

The formalism described above was applied to the same four-state biochemical cycle, with the same set of kinetic parameters, as used previously by Xie et al. (1994). At first, we examined the effect of the distribution in amplitude on flux by studying three types of amplitude distributions: the two-state, the evenly distributed, and the Gaussian-distributed (see Fig. 4). Then we tried to simulate the experimental results shown in Fig. 2.

For the two-state case, the amplitude of the RTN signal V in both the positive and the negative phases is fluctuating between Δ_1 and Δ_2. We assume that the two amplitudes are equally probable ($\alpha = ½$). Then the mean ($\bar{V}$) and the standard deviation (σ) of V are related to Δ_1 and Δ_2 as $\Delta_1 + \Delta_2 = 2\bar{V}$, $\bar{V} - \Delta_1 = \sigma$. These two equations determine the values of Δ_1 and Δ_2 at a given value of $\bar{V}$ and σ. For the evenly distributed case, the value of V is evenly distributed between $\bar{V} - l$ and $\bar{V} + l$, with a (constant) probability density of $1/(2l)$, where l can be shown to be equal to $3\sqrt{\sigma}$. The probability density curves for the two-state, the evenly distributed, and the Gaussian-distributed at $\bar{V} = 20$ and $\sigma = 5$ are shown in Fig. 5. The calculated transport fluxes at a mean potential of $\bar{V} = 20$ V/cm and a mean frequency of $f = 1$ kHz for the three types of amplitude distributions are plotted as a function of σ in Fig. 6. A few interesting results can be seen from the figure: 1) all three fluxes decrease at small σ, reach a minimum, and increase again as σ increases; 2) the flux is not sensitive to the type of distribution when σ is small (e.g., when $\sigma < 4$); 3) the two-state case is more sensitive to the fluctuation in the value of the amplitude than the Gaussian or the evenly distributed cases.

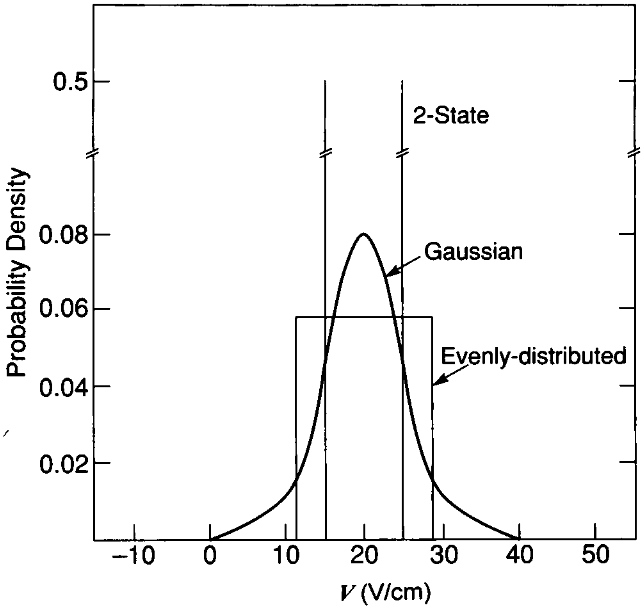

FIGURE 5 The probability densities of the variable V calculated for three types of distributions as discussed in the text at $\bar{V} = 20$ and $\sigma = 5$, where $\bar{V}$ is the mean and σ is the standard deviation of V.

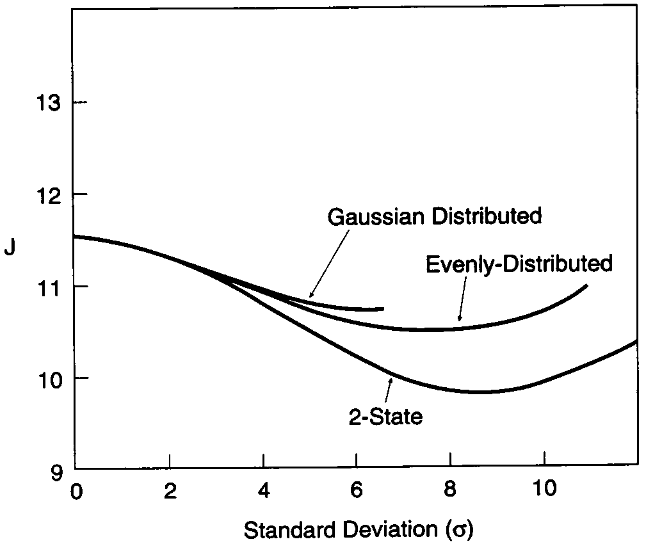

FIGURE 6 The flux (J) calculated as a function of σ for the four-state ECC model of Xie et al. (1994) in the presence of a RTN with a mean frequency of 1 kHz and a mean amplitude ($\bar{V}$) of 20 V/cm, for the three types of amplitude distributions discussed in the text. The same set of numerical parameters used by Xie et al. (1994) for the model was used in the calculation. J is in units of molecules per transporter per second.

In Fig. 7, the fluxes calculated at $f = 1$ kHz for the Gaussian-distributed case are plotted as a function of σ for a number of $\bar{V}$. The curves are found to be either monotonically decreasing or monotonically increasing, depending on the values of $\bar{V}$, in contrast to some of the curves found experimentally, as shown in Fig. 2.

DISCUSSION

The main purpose of the paper is to study how the dispersion (σ, or SD) in the amplitude of a RTN force field can influence the induced directional flux of a biological cycle. Electric activation of the RB^+-pumping mode of Na,K-ATPase was used as a model system for comparing experiment and theory. In theory, we first develop a general iteration procedure for the calculation of fluctuation-driven fluxes and then apply it to a simple four-state ECC transport model that has been used for the interpretation of electric activation phenomena in Na,K-ATPase. In general, the effect of dispersion in amplitude on the induced flux is not pronounced for the model. This result agrees well with experimental data shown in Fig. 2. However, there was disagreement in fine features between the experiment and the model analysis. For example, in Fig. 2, curves for the mean amplitude of 15 V/cm and 10 V/cm show nonmonotonic dependence on SD (σ). This is not seen in the simulated results for the four-state ECC model, as shown in Fig. 7. This minor disagreement means that the four-state ECC model is oversimplified, or that there are other properties of Na,K-ATPase that are not embodied in the ECC model. Another likely source of disagreement is experimental uncertainty. Because the amplitude dependence of transport efficiency is quite narrow, any experimental uncertainty may be exaggerated. Despite these minor discrepancies, our results have demonstrated unequivocally that Na,K-ATPase is capable of transducing energy contained in an RTN-field of considerable amplitude dispersion, and that the ECC model is a viable concept for understanding the electric activation of membrane ATPases.

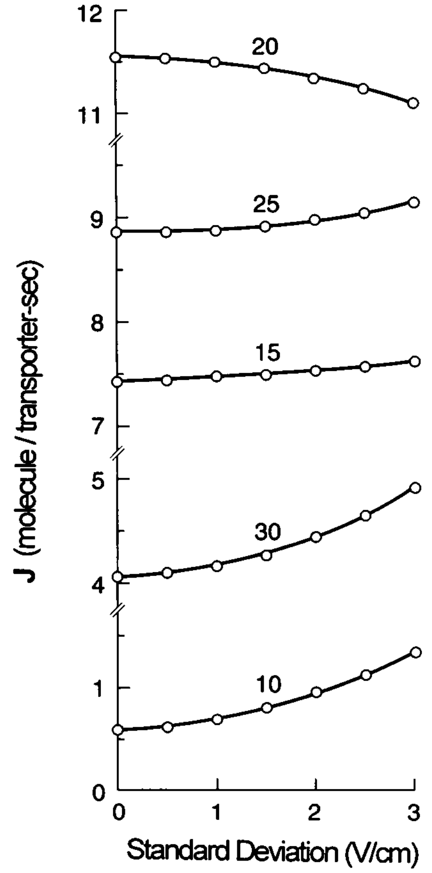

FIGURE 7 The RTN-induced flux of the four-state ECC model calculated as a function of σ for the Gaussian-distributed amplitudes at different mean values (as indicated by the numbers in units of V/cm). The same set of numerical parameters as used for the model of Xie et al. (1994) was used for the calculation.

There has been a surging interest in molecular mechanisms for the transduction of energy or signal in a randomly fluctuating force field (Astumian et al., 1987; Chen, 1987; Weaver and Astumian, 1990; Ertl, 1991; Xie et al., 1994; Chen and Tsong, 1994). Thermal ratchet has been discussed with respect to molecular motors in muscle or other biological systems (Astumian and Bier, 1994; Faucheux et al., 1995; Rousselet et al., 1994; Zhou and Chen, 1996). Stochastic resonance has also been discussed with respect to cellular processing of electric signal (Douglass et al., 1993; Bezrukov and Vodynaoy, 1995). In this respect, electroconformational coupling of Na,K-ATPase to RTN electric fields for ion pumping presents an interesting model system, both for detailed experimental study and for theoretical analysis.

Note added in proof: An article by R. Dean Astumian on Brownian motor, which discusses thermodynamics and kinetics of transduction of energy from fluctuating force field, is to appear in a May issue of *Science* (1997).

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