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The Response of Living Cells to Very Weak Electric Fields: The Thermal Noise Limit

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A physical model in which cells are considered as possible detectors of very weak periodic electric fields yields a general relation between cell size and both thermally induced fluctuations in membrane potential and the maximum change in membrane potential caused by an applied field. The simplest version of the model provides a broad-band estimate of the smallest applied electric field to which membrane macromolecules can directly respond (about 10^{-3} volt per centimeter). Much smaller fields (10^{-6} volt per centimeter) can be detected if there is a response in only a narrow band of frequencies or if signal averaging occurs through field-induced variation in the catalytic activity of membrane-associated enzymes. Both extensions of the simplest version remove the apparent violation of the thermal noise limit found in some experiments.

SIGNIFICANT BIOLOGICAL EFFECTS due to the exposure of cells to electric fields have been reported, including in vitro experiments involving isolated cells that demonstrated responses (for example, altered synthesis or transcription) to very weak fields (*1–5*). Controversy concerning the validity of weak field responses has arisen (i) because experimental reproduction of the effects has not always been achieved and (ii) because of the theoretical objection that the very small field magnitudes sometimes reported appear to be lower than allowed by thermal noise that causes randomization of cellular processes (*6*). The thermal noise limit is important because it is fundamental and also because other biological response mechanisms have a threshold close to this limit (*7*).

A cellular response to an external field implies that the applied field causes changes greater than those due to random fluctuating events ("noise"). Membranes have many sources of noise, including fundamental noise such as thermal noise, $1/f$ noise, and noise due to stochastic opening and closing of ion-conducting channels (*8*). Any cellular response to low fields must at least overcome the effects of thermal noise. Noise due to thermal fluctuations is well described by a fundamental physical theory. Random fluctuations in the transmembrane potential, $U(t)$, occur by virtue of a cell existing in a state of thermal equilibrium with its environment. As first observed by Johnson and explained by Nyquist, the thermally generated noise in an electrical resistance, R, is described by (*9*)

$$\overline{(\delta U)_{kT}^2} = 4RkT\Delta f \qquad (1)$$

where $kT = 4.3 \times 10^{-21}$ J ≈ 0.025 eV at 310 K is the product of the Boltzmann constant and the absolute temperature, Δf, is the frequency bandwidth within which information is sought, and the "bar" over $(\delta U)_{kT}^2$ denotes a time average. A worst case noise estimate can be made with a bandwidth Δf that is as large as possible (*10*). A cell membrane can be well represented by a parallel combination of membrane resistance R and membrane capacitance C, yielding an effective bandwidth $\Delta f = 1/(4RC)$ (*11*) and a broad-band estimate $\overline{(\delta U)_{kT}^2} = kT/C$.

For simplicity we first consider a spherical cell membrane of radius r_{cell}, for which the capacitance is $C \approx \epsilon_0 K_m 4\pi r_{cell}^2/d$. For the broad-band case

$$\overline{(\delta U)_{kT}^2} \approx kTd/(4\pi\epsilon_0 K_m r_{cell}^2) \qquad (2)$$

where ϵ_0 ($= 8.85 \times 10^{-12}$ C/V-m) is the permittivity of free space, K_m is the membrane's dielectric constant (typically 2 to 3), and d is the membrane's electrical thickness (about 50 Å). For a typical spherical mammalian cell (10-μm radius) the estimate is $(\delta U)_{kT} \equiv \sqrt{\overline{(\delta U)_{kT}^2}} = 2.8 \times 10^{-5}$ V at $T = 310$ K as the root-mean-square (rms) variation in the transmembrane potential due to thermal fluctuations. Imposition of a narrower bandwidth results in smaller $(\delta U)_{kT}$, so that this estimate represents a worst case.

An applied field E also causes changes in U. Although the general case of nonspherical geometry and finite membrane conductance gives rise to more complicated mathematical expressions, a basic response can be illustrated for the simple case of a perfectly insulating ($\sigma = 0$) spherical shell membrane, for which (*12*)

$$\Delta U_{max} \approx 1.5 E r_{cell} \qquad (3)$$

One can obtain the simplest estimate of E_{min}, the minimum field to which a cell can respond, by comparing ΔU_{max} to $(\delta U)_{kT}$. More specifically, in order for a cell to respond to E_{min}, we estimate that the applied field, E, must induce a change, $\Delta U_{max} \approx (\delta U)_{kT}$, that corresponds to a signal-to-noise ratio (S/N) of about 1, and yields (*10*)

$$E_{min} \approx \frac{2(\delta U)_{kT}}{3r_{cell}} = \frac{2}{3}\left[\frac{kTd}{4\pi\epsilon_0 K_m}\right]^{1/2}\frac{1}{r_{cell}^2} \equiv \frac{2A}{3}\frac{1}{r_{cell}^2} \qquad (4)$$

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For the same parameters used above, the factor A has the magnitude 2.8×10^{-10} V-m, and for a spherical cell with $r_{cell} \approx 10$ μm, the magnitude of E_{min} is 2×10^{-2} V/cm. This estimate is based on immediate responses of cell membrane molecules to the competition between the fundamental thermal noise and the applied field and is also applicable to dc fields.

Larger elongated cells, typical of many mammalian cells, can be represented by a cylindrical tube of length L_{cell} and radius r_{cell}, for which $\Delta U_{max} \approx L_{cell}E/2$. For such long cells the corresponding estimate is

$$E_{min} \approx \frac{2(\delta U)_{kT}}{L_{cell}} = 2\sqrt{2}\, A \frac{1}{r_{cell}^{1/2} L_{cell}^{3/2}} \quad (5)$$

For $L_{cell} = 150$ μm and $r_{cell} = 25$ μm, the estimate is $E_{min} \approx 8 \times 10^{-4}$ V/cm. Several experiments have revealed cellular responses at far smaller fields (Table 1).

We can estimate whether a limitation on the band ("narrow banding"), Δf, of responding frequencies can account for the observed low thresholds for detected fields. For example, inspection of the bovine fibroblast data (*3*) in Table 1 suggests an empirical identification of a "valley" or "window" with a corresponding frequency bandwidth $\Delta f \approx 10$ Hz. Although the actual cell membrane resistance, $R = \rho_m d/2\pi r_{cell} L_{cell}$, used in these experiments was not reported, we can make estimates on the basis of the extreme values of the membrane resistivity ρ_m and of Δf. Generally, ρ_m is in the range $10^5 \leq \rho_m \leq 10^7$ ohm-m (*13*). The same parameters as above for elongated cells lead to estimates of the minimum detectable electric fields, $E_{min,\Delta f}$:

$$E_{min,\Delta f} \approx \frac{4\sqrt{RkT\Delta f}}{L_{cell}} \approx 2\sqrt{2}\left[\frac{\rho_m k T d \Delta f}{\pi}\right]^{1/2} \frac{1}{r_{cell}^{1/2} L_{cell}^{3/2}} \quad (6)$$

of 3×10^{-5} to 3×10^{-4} V/cm for 100 Hz and 8×10^{-6} to 8×10^{-5} V/cm for 10 Hz, where the two choices of Δf are typical of the width of empirical "windows." Except for the combination of minimum ρ_m and Δf, these values are larger than the smallest experimental thresholds. At first glance, this result would suggest a possible violation of the thermal noise limit. In addition, there is no rationale beyond the experimental fit for invoking a narrow frequency band, Δf.

The paradox is resolved, however, if a cellular mechanism for "signal averaging" exists (*14*). Generally, if a periodic signal [the applied field, $E(t)$] of fundamental frequency f is applied for an exposure time τ, so that there are $N = f\tau$ cycles, S/N improves by the factor $(f\tau)^{1/2}$ (*15*). This extension of the previous broad-band estimate gives a minimum detectable field of

$$E_{min} \approx \frac{2A}{3} \frac{1}{r_{cell}^2 (f\tau)^{1/2}} \quad (7a)$$

for spherical cells and

$$E_{min} \approx 2\sqrt{2}\, A \frac{1}{r_{cell}^{1/2} L_{cell}^{3/2} (f\tau)^{1/2}} \quad (7b)$$

for elongated cells. The corresponding magnitudes for exposure at 1 kHz for 10^3 s are 2×10^{-5} V/cm (spherical cell, 10-μm radius) and 8×10^{-7} V/cm (elongated cell, 25-μm radius and 150-μm length). This estimate neglects the decrease in transmembrane potential changes due to the *RC* filtering that become important as higher frequencies are used. The small values of E_{min} predicted by Eq. 7 are in approximate agreement with the threshold conditions reported by others for observation of weak field effects (*3*). Still smaller fields may be detected if narrow banding is combined with signal averaging,

$$E_{min,\Delta f} \approx 2\sqrt{2}\left[\frac{\rho_m k T d \Delta f}{\pi}\right]^{1/2} \frac{1}{r_{cell}^{1/2} L_{cell}^{3/2} (f\tau)^{1/2}} \quad (8)$$

Recent experimental (*16*) and theoretical (*17*) work demonstrates that applied periodic electric fields can couple to membrane-associated, enzyme-catalyzed reactions through an electroconformational coupling mechanism. Many macromolecules, including membrane-bound enzymes, have conformational transitions that involve intramolecular movement of charge, or changes in dipole moments of the macromolecules, and are therefore sensitive to changes in the intense electric field, $\Delta E_m = \Delta U/d$, that exists across the membrane. Such field-induced conformational changes modulate enzyme activity. For example, a reaction that is poorly catalyzed may normally proceed at a negligible rate, but the rate may increase significantly upon a field-induced conformation change of the enzyme. Each cycle produces a "pulse" of product that may accumulate on one side of the membrane, effectively time-integrating the signal.

The enzyme can also transduce energy from the external field to drive the reaction it catalyzes (*17*). Electric field modulation of enzyme conformational transitions couples the "flow" of chemical free energy through the conversion of substrate into product to the "flow" of electrical free energy provided by the applied periodic field. An analogous situation exists for the more conventional detection of electrical signals by electronic devices: a high-input impedance amplifier controls the flow of electrical current with minimal loading of the source.

In general, enzyme electroconformational coupling is frequency specific. Optimal coupling is expected for frequencies between the characteristic inverse relaxation times. Typical expected rate constants for many enzymes lead to expectations of optimal coupling in the range 10^2 to 10^6 Hz, with $\Delta f > 10$ Hz, in general agreement with the results of both weak (*3*) and moderate (*16*) field experiments.

As a specific example, we consider a simple Michaelis-Menton–type enzyme embedded in a cell membrane (Fig. 1). If the applied periodic field is $E(t) = E_0\cos(2\pi ft)$, then for spherical cells the maximum change in transmembrane potential $\Delta U(t)$ is given by Eq. 3 with sinusoidal time dependence $\cos(2\pi ft)$, provided that $2\pi f$ is smaller than the inverse relaxation time of the double-layer capacitance. Thus, the maximum time-

Table 1. Comparison of experimental thresholds and the broad-band model predictions, with and without signal averaging. The electroconformational model generally provides narrow banding but cannot predict Δf for a particular cell membrane response because the particular enzyme parameters are not known. In the case of whole animals it is assumed that specialized sense organs involve electrically interconnected cells, such that the parameter L_{cell} can be associated with the length of the organ. Note especially the comparison between the bovine fibroblast data in the empirical "window" at about 1 to 10 Hz (*21*).

Frequency (Hz)	Time (s)	Minimum field (V/cm)		
		Experiment	Theory with averaging	Theory without averaging
		Chicken fibroblasts (4)		
1	8.6×10^4	2×10^{-5}	3×10^{-6}	9×10^{-4}
		Bovine fibroblasts (3)		
10^{-1}	4.3×10^4	1×10^{-2}	1×10^{-5}	9×10^{-4}
1	4.3×10^4	7×10^{-5}	4×10^{-6}	9×10^{-4}
10^1	4.3×10^4	5×10^{-5}	1×10^{-6}	9×10^{-4}
10^2	4.3×10^4	1×10^{-3}	4×10^{-7}	9×10^{-4}
10^3	4.3×10^4	2×10^{-2}	1×10^{-7}	9×10^{-4}
		Shark (2)		
~dc	~1	5×10^{-9}	4×10^{-9}	4×10^{-9}

varying transmembrane potential is $U(t) \approx 1.5 r_{cell} E_0 \cos(2\pi ft)$. This result may be substituted into the equations governing the kinetics of the reaction of Fig. 1, and the resulting equations solved for the enzyme state probabilities and the catalytic fluxes as a function of time (*18*). Product accumulates with time (Fig. 2). This mechanism is strongly frequency dependent (Fig. 3), with an optimal stimulation frequency of 3×10^3 Hz for the parameters used here. This narrow banding is generally expected, but the location and bandwidth depend on the particular enzyme.

In several experiments the observed threshold for a weak field response is well below the thermal noise limit estimated without including narrow banding or time averaging. However, the experimental thresholds are in reasonable agreement with the thermal noise limits when narrow banding and signal averaging are taken into account. Comparisons are given in Table 1.

The possibility also exists that an external field might act directly on isolated cytoplasmic macromolecules (*19*). Thus we also considered "free" biomolecules in solution as possible detectors of an applied field. The most favorable case for coupling to an applied field involves a linear macromolecule aligned parallel to *E*. One can obtain a simple estimate of the smallest detectable field by comparing thermal noise voltage fluctuations between the ends of the molecule with the end-to-end potential difference caused by a field. Any possible high effective conductivity along the molecule is neglected, so that the "noise" is governed entirely by the solution in which the molecule is dissolved. The resistance between the ends is dominated by ionic conduction in the surrounding electrolyte and is approximately equal to $\rho_e/4\pi a$, where ρ_e is the resistivity of the electrolyte and a is the effective radius of a hemispherical end-cap of the molecule. It is difficult to identify intrinsic broad-band widths or signal averaging mechanisms for an isolated molecule, so we used an experimentally determined frequency band Δf. We estimate $E_{min,free\ molecule} \approx \sqrt{2\rho_e kT\Delta f/\pi a}/L_{molecule}$, where $L_{molecule}$ is the length of the molecule, but do not include signal averaging. Using typical values ($L_{molecule} \approx 10^{-8}$ m, $a \approx 10^{-9}$ m, $\rho_e = 0.7$ ohm-m, and $\Delta f = 10$ Hz), we find that $E_{min,free\ molecule} \approx 1.4 \times 10^{-4}$ V/cm for exposure to a 100-Hz field, which is much greater than for membrane-associated macromolecules. We conclude that membrane constituents should be much better detectors of an applied field than are isolated molecules in solution. Interestingly, membrane receptors are also found to be more efficient detectors of chemical signals because of the geometric reduction of dimensionality of diffusion at the cell membrane surface (*20*).

We have shown that the lower limit for a minimum detectable field imposed by competition between an applied field and thermal noise is small. Other sources of noise that might lead to larger minimum detectable fields are not considered but should be investigated. However, the estimates presented here argue that concerns related to possible biological effects due to very weak environmental electric fields cannot be dismissed on the grounds of being swamped by thermal fluctuations.

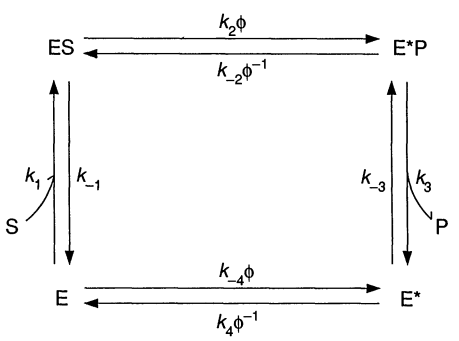

Fig. 1. A four-state mechanism for a membrane-bound enzyme E coupled to an electric field. For simplicity we have assumed that the catalyzed reaction substrate S = product P is not electrogenic and that the intrinsic rate constants k_i and k_{-i} do not depend on the field. The protein conformational changes may nevertheless involve the intramolecular movement of charge, so k_2 and k_{-4} are multiplied by a Boltzmann factor $\phi = \exp[qU(t)/2kT]$, and k_{-2} and k_4 are multiplied by $\exp[-qU(t)/2kT]$. The quantity q is the effective charge moved across the membrane (displacement charge) in the transition between E and E* and can be as large as six to ten elementary charges in the case of a voltage-gated Na^+ channel (*22*).

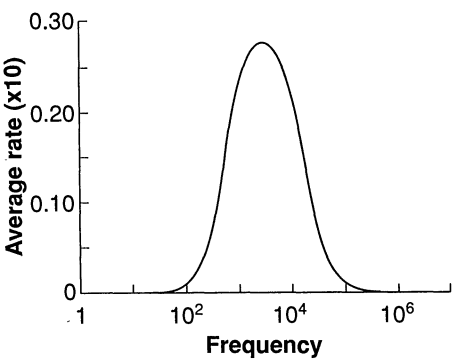

Fig. 2. Integrated accumulation of product P versus time that was calculated by inserting the time-dependent membrane potential $U(t) = U_0\cos(2\pi ft)$ into the differential equation resulting from the model of Fig. 1 and calculating the flux for the conversion of substrate S into P by a recently developed computational method (*18*). Both S and P were considered to be buffered to unit concentration. The values of other parameters are $k_1 = k_3 = 10^5\ s^{-1}$, $k_2 = k_4 = 10^3\ s^{-1}$, $k_{-1} = k_{-3} = k_{-2} = k_{-4} = 10^4\ s^{-1}$, $q = 5$ electronic charges, $r_{cell} = 5$ μm, $f = 3 \times 10^3$ Hz, and, in order to illustrate the effect, a relatively large field, $E_0 = 5$ V/cm. The primary requirement for the ac field to cause a net S → P flux is that the affinity of the enzyme for S must be different than the affinity for P, that is, a large interaction enery (*23*). If the affinity for S is greater, the induced flux is clockwise (Fig. 1); if the affinity for P is greater, the flux is counterclockwise.

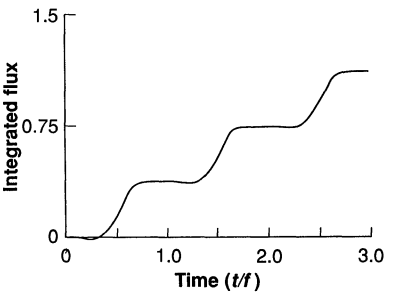

Fig. 3. The frequency dependence of the average rate of conversion of substrate S into product P by the periodic field. The parameters are the same as in Fig. 2, except that here $E_0 = 5 \times 10^{-3}$ V/cm. The units of the average product accumulation rate are molecules per second per enzyme molecule. A greater affinity difference (between S and P) for the enzyme results in a larger flux but also a broader bandwidth.

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Endothelin: A Novel Peptide in the Posterior Pituitary System

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Endothelin (ET), originally characterized as a 21-residue vasoconstrictor peptide from endothelial cells, is present in the porcine spinal cord and may act as a neuropeptide. Endothelin-like immunoreactivity has now been demonstrated by immunohistochemistry in the paraventricular and supraoptic nuclear neurons and their terminals in the posterior pituitary of the pig and the rat. The presence of ET in the porcine hypothalamus was confirmed by reversed-phase high-pressure liquid chromatography and radioimmunoassay. Moreover, in situ hybridization demonstrated ET messenger RNA in porcine paraventricular nuclear neurons. Endothelin-like immunoreactive products in the posterior pituitary of the rat were depleted by water deprivation, suggesting a release of ET under physiological conditions. These findings indicate that ET is synthesized in the posterior pituitary system and may be involved in neurosecretory functions.

ENDOTHELIN (ET) WAS ORIGINALLY characterized as a 21–amino acid vasoconstrictor peptide from endothelial cells, with a molecular weight of 2492 (*1*). The structure of ET is different from previously known bioactive peptides of mammalian origin (*1*). We have demonstrated a depolarizing action of ET on spinal neurons and the presence of ET in the porcine spinal cord (*2*). Autoradiographic studies in brain tissues also reported a nonvascular pattern of distribution of binding sites for ET (*3*). In addition, sarafotoxins, which are toxins from the burrowing asp (*Atractaspis engaddensis*) that have striking structural and functional similarities to ET, were reported to show high-affinity binding and hydrolysis of phosphoinositides in brain (*4*). These data suggest that ET may have an important role in neuronal function as a neuropeptide. However, the precise localization of ET in brain areas has not yet been elucidated. In the present study, we demonstrate a prominent localization of ET in the paraventricular and supraoptic nuclear neurons in the hypothalamus and in the neurohypophysis (posterior pituitary).

An antiserum was developed against [Arg^{14}]ET(15–21) conjugated with keyhole limpet hemocyanin (KLH) with *m*-maleimidobenzoyl-hydroxysuccinimide ester (*5*, *6*). ET(15–21) corresponds to the COOH-terminal region of ET (ETc), which is homologous in the ET family of proteins (*7*, *8*). Using the antiserum to ETc (ETc antiserum), we performed immunohistochemistry on porcine and rat brain and found strong ET-like immunoreactivity in the posterior pituitary systems of both species. The caudal part of the paraventricular nucleus (PVN) of the porcine hypothalamus (frontal section) is shown in Fig. 1A. A number of PVN neurons, mainly localized near the surface of the third ventricle, were densely immunostained for ET. The distribution of these ET-positive neurons in the PVN was similar to that of oxytocin-producing neurons, also reported to be confined adjacent to the third ventricle (*9*). In the supraoptic nucleus (SON), some neurons were strongly immunostained and others were negative in the same region (Fig. 1B) (*10*). In the rat, magnocellular neurons in PVN and SON were immunostained for ET (Fig. 1, C and D, respectively). Numerous dot-like immunoreactive structures were also found in the posterior pituitary (Fig. 1E). By contrast, antiserum that had been absorbed with ET showed negative immunostaining in the same region (Fig. 1F). These immunohistochemical data suggested the existence of ET-related peptides in these neurosecretory systems.

For the chemical identification of ET in the hypothalamus, we applied the peptide extract prepared from the porcine hypothalamic homogenates to a reversed-phase high-pressure liquid chromatography column and determined ET-like immunoreactivity in each fraction by radioimmunoassay (RIA) with ETc antiserum (*11*). There are three distinct ET-related genes encoding different ET-related peptides in the human genome: ET-1, identical to porcine and human mature ET; ET-2, [Trp^6, Leu^7]ET; and ET-3, [Thr^2, Phe^4, Thr^5, Tyr^6, Lys^7, Tyr^{14}]ET (*8*). The ETc structure is predicted to be preserved among these three peptides (*8*). The existence of three distinct ET-related loci has also been shown in the porcine and rat genomic DNAs (*8*). In the porcine hypothalamic extracts, however, the peaks of ET-like immunoreactivity emerged only at the elution time corresponding to that of ET-1 (Fig. 2), indicating that the major component in the porcine hypothalamus was ET-1.

Furthermore, to obtain direct evidence for the production of ET mRNA in PVN neurons, we examined porcine hypothalamus by in situ hybridization with a ^{35}S-labeled ET-1 complementary RNA probe. Neurons in the region of porcine PVN were heavily labeled, demonstrating the expres-

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