Diffusion of Calcium Ions in Retinal Rods

A Theoretical Calculation

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ABSTRACT The Fick diffusion equation is combined with the Langmuir adsorption isotherm and the relevant equations from the Gouy-Chapman theory of the electrical diffuse double layer to demonstrate that the effective diffusion coefficient of calcium ions, both in the cytoplasm of the rod outer segment and within the aqueous space bounded by the disk membrane, should be reduced by a factor of 10–100 because these ions adsorb to phospholipids present in the disk membrane.

INTRODUCTION

It has been proposed that excitation in vertebrate rods is mediated by a diffusible intracellular transmitter substance (Baylor and Fuortes, 1970). Although the identity of this putative transmitter is unknown, two specific hypotheses have received considerable attention. According to one hypothesis, photoactivated rhodopsin molecules modulate the activity of phosphodiesterases and thereby control the intracellular concentration of cyclic guanosine monophosphate (cGMP); the change in the concentration of cGMP then mediates excitation (e.g., Goridis et al. [1974] and Liebman and Pugh [1979]). We do not address the "cyclic nucleotide hypothesis" in this paper. According to the other hypothesis, the intracellular transmitter molecules are calcium ions (Yoshikami and Hagins, 1971; Hagins, 1972). Specifically, it has been proposed that the absorption of a photon by a rhodopsin molecule in the membrane of a disk causes the release of calcium ions into the cytoplasm of the rod outer segment, that these calcium ions diffuse from the disk to the plasma membrane, and that they block the channels passing the "dark current" through the plasma membrane. A consequence of this "calcium hypothesis" is that at least $10^2$ free calcium ions must enter the cytoplasmic space of the rod after a photon has been absorbed by a single rhodopsin molecule (Cone, 1973; Yoshikami and Hagins, 1973). Gold and Korenbrot (1980) and Yoshikami et al. (1980) have demonstrated that light induces an efflux of calcium from intact rod outer segments; the stoichiometry is $\sim 10^3$–$10^4$ calcium ions per activated rhodopsin. This observation suggests that the concentration of intracellular calcium changes either during the generation of
the light-induced electrical response, as predicted by the calcium hypothesis, or during the period of recovery from excitation. These changes in the concentration of calcium are probably accompanied by diffusional movements of calcium within the cytoplasm. In this paper we present quantitative arguments, based on the known adsorption constants of calcium with the phospholipids present in the disk membrane, that introduce constraints on the diffusion of calcium in the rod outer segment. We consider the diffusion of calcium ions both in the cytoplasm and within the aqueous space bounded by the disk membranes, the intradiskal space.

Bovine rod outer segments contain 45% phosphatidylethanolamine, 36% phosphatidylcholine, and 16% phosphatidylyserine, calculated as percent of the total phospholipid (Anderson et al., 1975); rod outer segments from rats and frogs have a similar composition (e.g., Daeman [1973]). At physiological pH, phosphatidylcholine and phosphatidylethanolamine are zwitterions and phosphatidylyserine has one net negative charge. Evidence from experiments with chemical labels suggests that most of the phosphatidylethanolamine and phosphatidylyserine are preferentially located on the outer or cytoplasmic monolayer of the disk membranes (Raubach et al., 1974; Smith et al., 1977; Crain et al., 1978; Dratz et al., 1979), although experiments with a phospholipase suggest that phosphatidylyserine may be more symmetrically distributed between the two monolayers (Drenthe et al., 1980). We assume in our analysis that the outer monolayer contains 15–30% and the inner monolayer 0–15% phosphatidylyserine.

We have measured the binding of calcium to bilayer membranes comprised of phosphatidylyserine, phosphatidylethanolamine, phosphatidylcholine, and mixtures of these lipids (McLaughlin et al., 1981). In all cases we were able to describe the adsorption by the Stern equation, which is a combination of the Langmuir adsorption isotherm, the Boltzmann relation, and the Grahame equation from the theory of the diffuse double layer. If macromolecules do not alter the adsorption of calcium to phospholipids and our measurements can be extrapolated to the bilayer component of the disk membranes, they indicate that there are 10–100 times as many calcium ions reversibly bound to the lipids on the outer surface of the disk membranes as there are free calcium ions in the cytoplasm. Similarly, there are 10–100 times as many calcium ions reversibly bound to the lipids on the inner surface of the disk membranes as there are free calcium ions in the intradiskal space.

One can draw two conclusions of biological relevance from the measurements of the adsorption of calcium to phospholipid bilayers. First, if the arguments of Cone (1973) and Yoshikami and Hagins (1973) are valid, the calcium hypothesis requires that $>10^3$ calcium ions per activated rhodopsin must be released to produce a net increase of $10^2$ free calcium ions. Second, if <1–10% of the calcium ions are free to diffuse, the effective diffusion coefficient of this ion will be markedly reduced. It is well known (e.g., Crank [1956]) that, when simultaneous diffusion and rapid, reversible binding of a solute occur in a homogenous medium, the movement of the solute may be described by the conventional Fick equation with the diffusion coefficient...
divided by a factor $1 + R$, where $R$ is the ratio of the bound to the free concentrations of the diffusing species. The analysis presented here illustrates that a similar relationship should hold for the diffusion of calcium, both in the cytoplasm of the rod outer segment and within the intradiskal space. The relevance of our analysis to the "calcium hypothesis" is considered in the Discussion section.

**Analysis**

**Model**

With reference to Fig. 1 we define:

$a$, radius of the disk membrane. The surface is assumed to be circular and flat, and any incisures in the disk are ignored (Cohen, 1972). (The incisures should probably be considered to obtain a realistic, three-dimensional solution to the diffusion equation. Our purpose here is not to solve such a diffusion equation but to point out that the diffusion coefficient of calcium in the spaces between and within the disks will be reduced because of the adsorption of this cation to phospholipids.)

$V$, volume of cytoplasm between two adjacent disks. The volume is defined by $r < a$, $-d/2 < z < d/2$. $V = \pi a^2 d$.

$Ca(z,r,t)$, concentration of calcium ions at a position $z,r$ at a time $t$. The units are moles per liter.

$PS(r,t)$, surface concentration of free phosphatidylserine molecules on the cytoplasmic side of the disk membrane. The units are moles per square centimeter.

$Ca-PS(r,t)$, surface concentration of calcium ions bound to phosphatidylserine. The units are moles per square centimeter.

For mathematical simplicity we assume that at a time $t = 0$ there is a release of $n$ calcium ions into the cytoplasm between two adjacent disks at $r = 0$, the center of the disk (Fig. 1). We consider other initial conditions in the Discussion.

**Fraction of Calcium Ions Free to Diffuse in the Cytoplasm**

A calcium ion in the aqueous phase adjacent to the membrane combines with a single phosphatidylserine molecule. The "intrinsic" association constant, $K_{Ca}$, is:

$$
K_{Ca} = \frac{Ca-PS(r,t)}{Ca(\pm d/2,r,t)PS(r,t)},
$$

where $Ca(\pm d/2,r,t)$ is the concentration of free calcium ions in the aqueous phase at the membrane-solution interface a distance $r$ from the origin at a time $t$. This concentration is substantially different from the concentration of calcium in the plane $z = 0$, $Ca(0,r,t)$, because the negatively charged phosphatidylserine molecules tend to concentrate the calcium ions in the aqueous "diffuse double layer" near the disk membranes (e.g., McLaughlin [1977]).

We note that the mean time for diffusion in the $z$ direction is much less
than the mean time for diffusion in the \( r \) direction. The mean diffusion time may be calculated in an approximate manner by dividing the square of the distance by the diffusion constant (Einstein, 1956). The distance \( d \) is \( \sim 150 \) Å (Gras and Worthington, 1969; Korenbrot et al., 1973), whereas the distance \( a \) is \( \sim 1-3 \) μm for rat and frog rods (Cone, 1973). If \( D = 6 \times 10^{-6} \text{cm}^2/\text{s} \) (Hodgkin and Keynes, 1957), diffusional equilibrium will occur in the \( z \) direction in less than a microsecond but take longer than a millisecond to occur in the \( r \) direction. It is thus reasonable to assume that the electrochemical potential of a calcium ion is a constant in the \( z \) direction for the times that we consider (\( t > 10^{-6} \) s). If the standard chemical potential and the activity coefficient of calcium are independent of \( z \), the Boltzmann relation is valid:

\[
Ca(z,r,t) = Ca(0,r,t) \exp\left(-\frac{2F\psi(z)}{RT}\right),
\]

where \( \psi(z) \) is the electrostatic potential at \( z \) relative to the value at \( z = 0 \). In particular, the free concentration of calcium in the aqueous phase at the surface of the disk membrane is:

\[
Ca(\pm d/2,r,t) = Ca(0,r,t) \exp\left(-\frac{2F\psi(d/2)}{RT}\right),
\]

where \( \psi(d/2) \) is the surface potential. We assume that \( \psi(d/2) \) may be calculated from the classical Gouy-Chapman theory of the diffuse double layer (see Appendix) and that the phosphatidylserine molecules on the cytoplasmic surface are distributed uniformly over the disk membrane. The evidence for the latter assumption is far from conclusive (Massari et al., 1978; Favre et al., 1979; Sklar et al., 1979 a and 1979 b). The surface concentration of free

![Diagram of two adjacent disks in the cytoplasm of a rod cell, drawn to illustrate the coordinate axes. The diagram is not to scale: the thickness of the membrane is \( \sim 50 \) Å; the thickness of the intradiskal space is \( \sim 20 \) Å; the thickness of the cytoplasmic space, \( d \), is \( \sim 150 \) Å; and the radii of rat and frog disks, \( a \), are 1 and 3 μm, respectively.](image)
phosphatidylserine molecules, \( PS(r,t) \), is less than the total surface concentration, \( PS^{tot} \), because these molecules combine with alkali metal and alkaline earth cations in the cytoplasm. Thus,
\[
PS^{tot} = PS(r,t) + Ca-PS(r,t) + Mg-PS + Na-PS + K-PS,
\]
where \( Mg-PS, Na-PS, \) and \( K-PS \) are, respectively, the surface concentrations of the magnesium, sodium, and potassium complexes with phosphatidylserine. The \( Mg-PS \) term may be ignored because the total concentration of Mg in the cytoplasm (Szuts and Cone, 1977) is much less than the total equivalent volume concentration of phosphatidylserine, \( (2/d)PS^{tot} = 50-100 \text{ mM} \). The Stern equation can describe adequately the simultaneous adsorption of calcium and the alkali metal cations to phospholipid bilayer membranes containing phosphatidylserine (McLaughlin et al., 1981). The values of the intrinsic association constants for the monovalent cations are \( 0.6 \text{ M}^{-1} \) for \( Na-PS \) and \( 0.15 \text{ M}^{-1} \) for \( K-PS \) (Eisenberg et al., 1979). If we take reasonable estimates for the concentrations of these cations in the cytoplasm, \( [K] = 0.15 \text{ M} \) and \( [Na] = 0.015 \text{ M} \), and note that the Gouy equation (Eq. A3) predicts the surface potential cannot be more negative than \(-60 \text{ mV}\), it follows that deleting the \( Na-PS \) and \( K-PS \) terms in Eq. 4 results in a \(<25\% \) overestimate of \( PS(r,t) \). If we assume that \(<10^4 \) calcium ions are released per activated rhodopsin and recall that there are \( 10^6-10^7 \) phosphatidylserine molecules in a rat or frog disk, it follows that \( Ca-PS(r,t) < 0.1 \text{ PS}^{tot} \) once diffusion has proceeded a radial distance \( r > 0.3a \). Thus, we assume that
\[
PS(r,t) = PS^{tot}
\]
for most of the diffusion process. Combining Eqs. 1, 3, and 5 we obtain
\[
Ca-PS(r,t) = K_{Ca}Ca(0,r,t)\exp\left(-2F\Psi(d/2)/RT\right)PS^{tot}.
\]
The important feature of Eq. 6 is that the equivalent volume concentration of bound calcium ions, \( (2/d)Ca-PS(r,t) \), is proportional to the free concentration of calcium ions in the plane at \( z = 0, Ca(0,r,t) \).

**Diffusion Process**

In the absence of any adsorption of calcium ions Fick’s second law will be valid:
\[
\frac{\partial Ca(z,r,t)}{\partial t} = D\nabla^2 Ca(z,r,t),
\]

1 We ignore the adsorption of calcium to the zwitterionic lipids phosphatidylcholine and phosphatidylethanolamine. The intrinsic association constants of these lipids with calcium (3 M\(^{-1}\)) are one-fourth the intrinsic association constant of phosphatidylserine with calcium (12 M\(^{-1}\)) (McLaughlin et al., 1981). The Stern equation can describe satisfactorily the adsorption of calcium to membranes formed from mixtures of phosphatidylserine and these zwitterionic lipids (McLaughlin et al., 1981), and it is trivial to extend Eq. 6 to include the binding of calcium to these lipids. Inasmuch as their surface concentration is 2-6 times that of phosphatidylserine in the outer monolayer of a disk membrane, about a factor of 2 error is involved in ignoring this adsorption. The inclusion of these binding terms would only strengthen our conclusion that the adsorption of calcium to lipids in the disk membrane can dramatically reduce the effective diffusion coefficient of calcium in the cytoplasm.
where \( D \) is the diffusion coefficient of calcium ions free in the aqueous solution (\( D = 6 \times 10^{-6} \) cm\(^2\)/s; Hodgkin and Keynes, 1957). The Laplacian is taken only in the \( r \) direction,

\[
\nabla^2 = \frac{\partial^2}{\partial r^2} + \frac{1}{r} \frac{\partial}{\partial r},
\]

because, as noted in Eq. 2, the electrochemical potential of calcium ions is assumed to be constant in the \( z \) direction. However, calcium ions do adsorb to and desorb from phosphatidylserine molecules (McLaughlin et al., 1981). The change in the concentration of calcium ions contained in an infinitesimal volume located at the position \( z, r \) in the time interval between \( t \) and \( t + \Delta t \), \( \Delta t > 10^{-8} \) s, results not only from the net flux of calcium entering or leaving the volume in the radial direction, a term given by the Laplacian in Eq. 7, but also from the release or uptake of calcium ions by the binding sites on the surface of the membrane. It can be shown that the partial derivative of the concentration of calcium ions at position \( z, r \) and time \( t \) with respect to time is given by:

\[
\frac{\partial C_a(z, r, t)}{\partial t} = \frac{1}{4 \pi \varepsilon_0} \frac{\partial C_a-PS(r, t)}{\partial t} \int_0^{d/2} \exp\left[-\frac{2F\Psi(z)}{RT}\right] dz.
\]

Inserting the Boltzmann relation, Eq. 2, into Eq. 8 and integrating from zero to \( d/2 \) to remove the dependence on \( z \), we obtain:

\[
I \frac{\partial C_a(0, r, t)}{\partial t} = IDV^2 C_a(z, r, t) - \frac{\partial C_a-PS(r, t)}{\partial t} \int_0^{d/2} \exp\left[-\frac{2F\Psi(z)}{RT}\right] dz.
\]

where:

\[
I = \frac{(2/d)}{\int_0^{d/2} \exp\left[-\frac{2F\Psi(z)}{RT}\right] dz}.
\]

The integral is evaluated in the Appendix. The calculated value of \( I \) is 3.9 if all of the phosphatidylserine is on the cytoplasmic surface of the disk and 1.7 if half of the phosphatidylserine is on the cytoplasmic surface of the disk.

We now return to Eq. 9 and note that it is formally identical to the expression that describes the simultaneous diffusion and binding of calcium in a homogeneous medium (e.g., Crank [1956], Eq. 8.3). The effective volume concentration of bound calcium is \((2/d)C_a-PS(r, t)\), and the average concentration of free calcium is \(C_a(0, r, t)\). Blaustein and Hodgkin (1969) have solved the problem of concomitant diffusion and binding in a membrane lined cylinder for precisely our initial conditions and may be consulted for a discussion of the solutions for their particular boundary conditions. We prefer not to specify precise boundary conditions here, but merely to complete the derivation of the diffusion equation. We now assume that the binding reaction,
Eq. 6, may be considered to be at equilibrium throughout most of the diffusion process. The combination of Eqs. 6 and 9 yields

$$\frac{\partial Ca(0,r,t)}{\partial t} = \frac{D \nabla^2 Ca(0,r,t)}{1 + (2/d)K_{ca}\exp[-F\psi(d/2)/RT]PS^{tot}(1/I)}.$$  

(11)

Note that Eq. 11 is identical in form to Fick's second law, Eq. 7, with the aqueous diffusion coefficient of calcium, $D$, divided by the constant factor

$$1 + (2/d)K_{ca}\exp[-2F\psi(d/2)/RT]PS^{tot}(1/I) = D/D_{eff},$$  

(12)

where $D_{eff}$ is the effective diffusion coefficient. To calculate this term we need to know the value of the surface potential. If the cytoplasmic surface of the disk membrane contains 15–30% phosphatidylserine and the cytoplasm contains 0.15 M KCl and negligible concentrations of divalent cations, the Gouy equation from the theory of the diffuse double layer (Eq. A3) predicts that the surface potential will be $-35$ (15% PS) to $-60$ (30% PS) mV. The value of $I$ is calculated to be in the range from 1.7 (15% PS) to 3.9 (30% PS). The value of $K_{Ca}$ is measured to be $12 \text{M}^{-1}$ (McLaughlin et al., 1981). The effective volume concentration of phosphatidylserine, $(2/d)PS^{tot}$, is 50 (15% PS) to 100 (30% PS) mM. Thus, the diffusion coefficient is reduced by a factor of 7–31.

**DISCUSSION**

There is indirect evidence that calcium is involved in the process of visual excitation in vertebrate rods. Calcium appears to be located mainly within the disks in intact rods (Fishman et al., 1977; Schnetkamp, 1979) and electrophysiological experiments have shown that an increase in the cytoplasmic concentration of calcium ions mimics some aspects of excitation, whereas the introduction of a calcium sequestering agent tends to desensitize the photoreceptors (Brown et al., 1977; Hagins and Yoshikami, 1977). We suggest, on the basis of the analysis presented above, that some of the arguments raised against the calcium hypothesis in the past should now be reconsidered. For example, the time for free diffusion can be estimated by dividing the square of the distance by the diffusion constant, and it has long been recognized that

$$2 \text{ More generally we should write, in Eq. 9, that } \frac{\partial Ca-PS(r,t)}{\partial t} = k_1Ca(d/2,r,t)PS^{tot} - k_2Ca-PS(r,t),$$

where $k_1$ and $k_2$ are the rate constants for the reaction. We are justified in assuming that this reaction is essentially at equilibrium during the diffusion process if $k_1(2/d)PS^{tot} + k_2 \gg D/a^2$. When we divide through the inequality by $k_2$, we note that $k_1/k_2 = K_{Ca} = 12 \text{M}^{-1}$ (McLaughlin et al., 1981) and that $(2/d)PS^{tot}$, the equivalent volume concentration of phosphatidylserine in the cytoplasm, is in the range from 0.05 M (the cytoplasmic monolayer of the disk consists of 15% phosphatidylserine) to 0.1 M (the cytoplasmic monolayer of the disk consists of 30% phosphatidylserine). Thus, the condition becomes $k_2 \gg D/a^2$. The rate constant is known for only one divalent cation and one lipid: for cobalt and phosphatidylethanolamine the life time of the complex is $3 \times 10^{-6}$ s (McLaughlin et al., 1978) or the rate constant is $3 \times 10^5$ s$^{-1}$. If the rate constant for the calcium-phosphatidylserine complex is not slower by two orders of magnitude, our approximation is valid.
“if the transmitter is an ion or small molecule with a diffusion coefficient $D \sim 10^{-5}\, \text{cm}^2/\text{s}$, then most of the transmitter should diffuse to and contact the plasma membrane of a rod within 1–10 ms after being released” by an activated rhodopsin (Cone, 1973). This time is about two orders of magnitude shorter than the times-to-peak of the receptor potentials elicited by dim stimuli in rat and frog rods, about 200 and 500 ms, respectively (Cone, 1973). The time-to-peak of the receptor potential could be much longer than the time for free diffusion, either because of reactions that intervene between rhodopsin activation and transmitter release or because of reactions that occur at the plasma membrane. A detailed kinetic scheme has been presented that can account for the shape of the wave form observed in turtle cones upon photoactivation (Baylor et al., 1974a and 1974b; Baylor and Hodgkin, 1974).

If, however, calcium is the intracellular transmitter, then the simultaneous diffusion-adsorption process described above can itself account for the long time-to-peak of the receptor potential. Specifically, our analysis indicates that the diffusion coefficient of calcium should be reduced by a factor of 7–31 because of binding of this ion to phosphatidylserine. If the adsorption of calcium to the zwitterionic lipids present in the disk membrane is included in the analysis, the diffusion coefficient decreases further by a factor of 2. Any binding of calcium to proteins, sugars, ATP, etc., and any active transport of calcium into the disks would further reduce the effective diffusion coefficient. Thus, the effective diffusion coefficient of calcium in the cytoplasm of the rod outer segment must be at least one to two orders of magnitude lower than the diffusion coefficient of calcium ions free in an aqueous solution. The observation that the time-to-peak of the receptor potential increases with an increase in the radius of the rods obtained from various species (e.g., Cone [1973]) is clearly consistent with the hypothesis that the initial rise in the visual response is limited by a simultaneous diffusion-adsorption process.

Elegant experiments on the responses of toad rods to single photons led Baylor et al. (1979) to conclude that a diffusion process does not limit the initial rise of the electrical response to light. They argued as follows. “Several authors have suggested that a diffusion process may limit the initial rise of the visual response to light (Ives, 1922; Cone, 1964; Rushton, 1965; Kelly, 1971). If a significant component of the delay arose from radial diffusion of internal transmitter from the site of absorption in a disk to the outer plasma membrane, then the form of the quantal event should depend on the radial position at which the isomerization occurred. In particular, absorption of a photon near the perimeter would give a large response with a little delay, while absorption near the centre would give a smaller and slower response. In our experiments a given cell’s quantal responses varied only slightly in time-course and amplitude between trials. This, together with the points mentioned by Baylor et al. (1974), suggests that diffusion is not a major source of delay in the photoresponse, although it certainly must make some contribution.” The argument of Baylor et al. (1979) is correct, of course, if one assumes that the photoactivation process produces an instantaneous source of the transmitter in the cytoplasm. The choice of a different initial condition, however, indicates
that the experiments of Baylor et al. (1979) do not rule out a diffusion process.

If diffusion of the transmitter substance in the intradiskal space is the rate-limiting step, the time-to-peak for the visual response to a single photon will be essentially independent of the location of the release site. The diffusion of transmitter within the disks is rate limiting if

$$\frac{a^2}{D_{\text{eff}}} < \frac{b^2}{D_{\text{eff}}}$$

where \(a\) is the radius of the disk, \(D_{\text{eff}}\) is the effective diffusion coefficient of the transmitter substance in the cytoplasm, \(b\) is the average distance that a transmitter molecule must diffuse in the intradiskal space before it can move into the cytoplasm, and \(D_{\text{eff}}\) is the effective diffusion coefficient of the transmitter substance in the intradiskal space. The value of \(D_{\text{eff}}\) for calcium is given by Eq. 12. A maximum value of \(D_{\text{eff}}\) for calcium may be calculated by assuming that the inner monolayer of the disk contains a negligible concentration of phosphatidylserine and other charged molecules. If the surface potential of the inner monolayer of the disk membrane is zero, the diffusion coefficient is reduced by the factor

$$1 + \frac{(2/g)K'_{\text{Ca}}(PC + PE)}{D/D_{\text{eff}}},$$

In this expression \(D\) is the diffusion coefficient of calcium ions free in the aqueous phase within the disk, \(g \approx 20 \text{ Å}\) is the thickness of this intradiskal space (Chabre and Cavaggioni, 1975), \(K'_{\text{Ca}} = 3 \text{ M}^{-1}\) is the intrinsic association constant of calcium with phosphatidylcholine and phosphatidylethanolamine (McLaughlin et al. 1981), and \((PE + PC) = 1/70 \text{ Å}^2\) is the surface concentration of the zwitterionic lipids in the inner monolayer of the disk membrane. Thus, \(D_{\text{eff}} < 0.1D\). The presence of negatively charged molecules such as phosphatidylserine or rhodopsin on the inner monolayer will further reduce \(D_{\text{eff}}\). If the inner and outer surface potentials are similar, proportionately more calcium ions will be adsorbed to phospholipids in the intradiskal space than in the cytoplasmic space, and the effective diffusion coefficient of calcium in the intradiskal space will be reduced more than the effective diffusion coefficient of calcium in the cytoplasmic space, because the thickness of the intradiskal space, \(g\), is about one-tenth the thickness of the cytoplasmic space, \(d\). Thus, \(D_{\text{eff}}\) could be significantly less than \(D_{\text{eff}}\), and inequality 13 could be satisfied. A more detailed diffusion calculation would involve assumptions about the number of calcium ions released per activated rhodopsin, the number of calcium ions contained within a disk, the possible diffusion of the activated rhodopsin during the release process, the rate at which calcium is pumped into the disks, etc. The available experimental data are not adequate.
to test critically the notion that the diffusion-adsorption of calcium within disks may be rate limiting. The responses of rods to the adsorption of single photons are sufficiently noisy to vitiate exact estimates of the variability of the latency (Baylor et al., 1979).

We conclude by noting that a consideration of the adsorption of calcium to phospholipids not only removes some of the objections to the simplest form of the "calcium hypothesis" but also places additional constraints on this hypothesis. For example, if <1–10% of the calcium in the cytoplasm is free to diffuse, then \(10^3\)–\(10^4\) calcium ions must be released to produce the \(>10^2\) free calcium ions required by the transmitter hypothesis. Gold and Korenbrot (1980) and Yoshikami et al. (1980) have detected, in the aqueous phase adjacent to the rods, a release of \(~10^3\)–\(10^4\) calcium ions per activated rhodopsin.

**APPENDIX**

The integral in Eq. 10 is evaluated in the following manner. We first note the mathematical identity

\[
2 \exp(-F\psi(z)/2RT) \cdot \sinh (-F\psi(z)/2RT) + 1 = \exp(-F\psi(z)/RT).
\]

(A1)

Eq. A1 is substituted into Eq. 10 to obtain

\[
I = \frac{2}{d} \int_0^{d/2} \left\{ 2 \exp(-F\psi(z)/2RT) \cdot \sinh(-F\psi(z)/2RT) + 1 \right\} \cdot \left\{ \exp(-F\psi(z)/RT) \right\} dz.
\]

(A2)

We note that when the ratio of the concentration of divalent to monovalent cations at \(x = 0\) is low, the simple Gouy relationship from the theory of the diffuse double layer may be invoked (e.g., Grahame, [1947]):

\[
\sinh (-F\psi(z)/2RT) = \frac{A\sigma(z)}{\sqrt{C}},
\]

(A3)

where \(A = 1/(8\epsilon_0\epsilon_a N k T)^{1/2}\), \(\epsilon_a\) is the dielectric constant of water, \(\epsilon_0\) is the permittivity of free space, \(N\) is Avogadro's number, \(k\) is Boltzmann's constant, \(T\) is the absolute temperature, \(C\) is the bulk aqueous concentration of monovalent cations, and \(\sigma(z)\) is the net surface change density (charge on the surface minus the space charge in the diffuse double layer between \(d/2\) and \(z\)).

feasible and concluded that "the binding of Ca\(^{2+}\)" in photoreceptor membranes takes place primarily through the phosphate groups of phospholipids." Other measurements are discussed by Schnetkamp (1979) and Kaupp et al. (1979). Our measurements on phospholipid bilayer membranes formed from phosphatidyleserine, phosphatidylcholine and mixtures of phosphatidylethanolamine with phosphatidylserine (McLaughlin et al., 1981) demonstrate that the adsorption of calcium to these bilayer membranes is relatively independent of temperature, at least in the range between 15° and 45°C. If these measurements can be extrapolated to disk membranes, they suggest that the temperature dependence of the time-to-peak must arise from some phenomenon other than the adsorption of calcium ions to lipids, possibly from the temperature dependence of the calcium pumps that are postulated to exist in the disk and plasma membranes.
Gauss' law states that
\[ \sigma(z) = -\varepsilon_0 \frac{\partial \psi(z)}{\partial z}. \] (A4)

Combining Eqs. A1–A4 yields
\[ I = \left( \frac{2}{d} \right) \left( \frac{2}{3} \right) \left( \exp\left[ -3F \psi(d/2)/2RT \right] - 1 \right) \] 
\[ + 2 \left( \exp\left[ -F \psi(d/2)/2RT \right] - 1 \right) + 1, \] (A5)

where \( 1/\kappa \) is the Debye length. If the temperature, \( T \), is 20°C and the concentration of monovalent cations in the cytoplasm of the rod outer segment, \( C \), is 0.15 M, the Debye length is
\[ \frac{1}{\kappa} = \left( \frac{\varepsilon_0 \kappa T}{2e^2 NC} \right)^{1/2} = 7.9 \text{ Å} \] (A6)

If the cytoplasmic surface of the disk contains 30% phosphatidylserine and each lipid occupies 70 Å², the charge density is \( \sigma = -0.3/70 \text{ Å}^2 \). From Eq. A3 the potential at the surface of the membrane, \( z = d/2 \), is \( \psi(d/2) = -60 \text{ mV} \). If the cytoplasmic surface of the disk contains only 15% phosphatidylserine \( \psi(d/2) = -35 \text{ mV} \). Inserting these values of the surface potential into Eq. A5, we deduce that \( 1.7 < I < 3.9 \).

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