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EARLY RECEPTOR POTENTIAL GENERATING MECHANISM IN VERTEBRATE RETINA

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ABSTRACT

W. K. Wang (1975). *Early receptor potential generating mechanism in vertebrate retina.* Bull. Inst. Zool., Academia Sinica, **14**(1): 19-26. A charge separation and subsequent charge spreading model is used to explain the generating mechanism of early receptor potential in the vertebrate retina. The R1 is from direct charge separation. The R2 is from ion absorption and subsequent ion migration, and this produce a diffused electrical double layer. Special attention is paid to the rod envelope which is a good capacitor. Because of this capacitor, the electric field due to charges separation needs only to extend to the rod envelope to charge up the capacitor, the induced charges outside the envelope will produce a transient potential which can be measured a long distance away. The absorbed ions are hydrogen ions, the OH ion to be the transmitter for the late receptor potential is also proposed.

The early receptor potential (E. R. P.) was first described by Brown and Murakami[1] in 1964. In the same year Cone[2] had shown that its magnitude is proportional to the number of the rhodopsins bleached. Its relation to temperature, pH and different ions has been shown by Brindley *et al.*[3] and Pak *et al.*[4]. Goldstein[5] found that most of the extracellular response originated from the cones. Toyoda *et al.*[6] proved that E. R. P. is not the cause for the late receptor potential (L. R. P.). Murakami and Pak[7] investigated the relationship between the extracellular recording and the intracellular recording and concluded that the rod outer segment envelope acts like a capacitor. Recently Rüppel and Hagins[8] measured the E. R. P. spatial distribution from a rod.

The two responses of E. R. P. which appear between the photon absorption and the appearance of the L. R. P. are called R1 and R2. They do not depend on the living cell; even after the treatment by formaldehyde the respose still can be measured[3]. The origin of this response was first suggested to be a result of some charge movement through some asymmetric structure in the rod or cone outer segment[9]. Brindley *et al.*[3] proposed independently an equivalent circuit which is based on the structure of the cone outer segment and a similar asymmetric concept.

To explain the spatial distribution of E. R. P. along the rod outer segment, Rüppel and Hagins[8] have suggested that the charge displacement is on the rod envelope. This model will explain some of the R1 results. For R2, the pH and the temperature dependence are two important requirements that any good model must meet.

In the present paper, a new mechanism

which can explain all the available data will be presented. Some experiments are also suggested which distinguish this model from other models.

SOME GENERAL CONSIDERATION

Let us start with some simple examples. For a dipole, the potential will be positive near the positive charge and be negative near the negative charge. The concept of a dipole as consisting of two point charges is theoretical. In reality, a dipole usually has a volume which is positively charged and a volume which is negatively charged. The size of the positively charged volume does not have to be of the same magnitude as the negatively charged one. For example, in the water molecule, both hydrogen atoms are situated at the positive pole and the oxygen atom is at the negative pole. For large molecules, the charged volume may be very large, therefore there exists a situation in which the negative charge is confined to a very small volume, surrounded by a spreaded positive charge.

When we think of separation of opposite charges, what comes to mind is a dipole, a point charge dipole, and should there be a current, it must be from the positive charges toward the negative charges. However, a transient current may also be produced by spreading out of these poles. A common example is the electroscopic measurement of the charge built up in a plexiglas after it is rubbed with wool; the current flows from the plexiglas toward the electroscope which has no contact with the wool.

Here I shall present a special situation which will spread a transient potential into a large volume (Fig. 1). After the separation of charge, the positive pole will not be confined to the small volume A, but it will spread along the conductor toward the capacitor. The fraction of the original charge $+q$ that will stay in volume A is a funtion of r/d, where d is the distance between the two poles and r is the distance between the volume A and the capacitor. If $r \gg d$, most of the charge will stay on the volume A. If $d \gg r$, most of the charge will spread toward the capacitor. The charge $+q'$ on the right side of the capacitor will induce $-q'$ on the left side of the capacitor; the final situation is as shown in Fig. 1C. Because of this separation of charge, another separation of charge is induced at the left side of the cap-

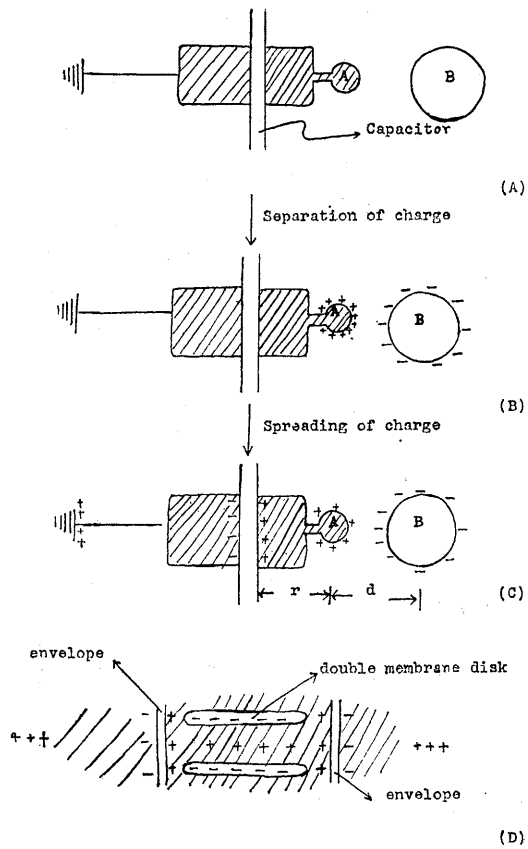

Fig. 1. The shaded areas are conductors. (A) is the situation before the charge separation. (B) is the situation right after the charge separation. (C) is the final situation after the charge spreading. (D) is a schematic drawing of a short segment of rod outer segment. The cytoplasm is the positive pole and the conductor, the double membrane disks are the negative pole, and the envelope is the capacitor.

acitor. Therefore if we measure the potential at the left side of the capacitor immediately after the original separation of charges at A and B, a transient positive potential can be measured. The magnitude of the potential will depend on the conductance and the r/d.

The ultrastructure of the outer segment of rod and cone have been resolved by electron microscopy[10,11]. The outer segment consists of an envelope and a pile of double membrane disks which are photo-sensitive. For a cone the double membrane is actually a folding of the envelope. For a rod the double membrane disks are pinched off the envelope and there is about 100 Å space between the rod double membrane disks and the envelope[10].

R1 GENERATING MECHANISM

After a photon is absorbed by the rhodopsin, the 11-cis retinal isomerize to all-trans retinal. There is probably a charge separation process, and if some positive charge suddenly appear in the highly conducting cytoplasm, the negative charges remain on the dielectric double membrane disks (see Fig. 1). A dipole is produced, in which the interdisk cytoplasm is the positive pole and the double membrane disk is the negative pole. Because the cytoplasm has a high conductance, the positive charges tend to spread along the cytoplasm toward the envelope. The envelope behaves as a good capacitor[7] and we have the scheme in Fig. 1 D. The cytoplasm is the positive pole and conductor, the double membrane disks is the negative pole, the envelope is the capacitor and the outside ionic solution is the ground conductor. We may approach this mechanism more analytically by the method of image[12]. The positive charge is in the cytoplasm with distance r fom the double membrane disk surfaces and the negative charge are right on the double membrane disks.

For any charge q in the cytoplasm with distance γ from the membrane with high dielectric constant the image charge is at distance $\gamma=-\gamma$ with magnitude $q'=-q\ (\epsilon_2-\epsilon)/(\epsilon_2+\epsilon)$; ϵ is the the dielectric constant of cytoplasm. ϵ_2 is the dielectric constant of the membrane, and for $\epsilon_2 \gg \epsilon$, we have $q' \simeq -q$ $\gamma'=-\gamma$. For the negative charge, because $\gamma'=-\gamma=0$, q and its image $-q$ coincide and cancel with each other. For the positive charge q, we have $q'=-q$, $\gamma'=-\gamma$. Thus we may calculate the voltage and electric field at the rod envelope, contributed from the positive charge and its image. (Fig. 2A).

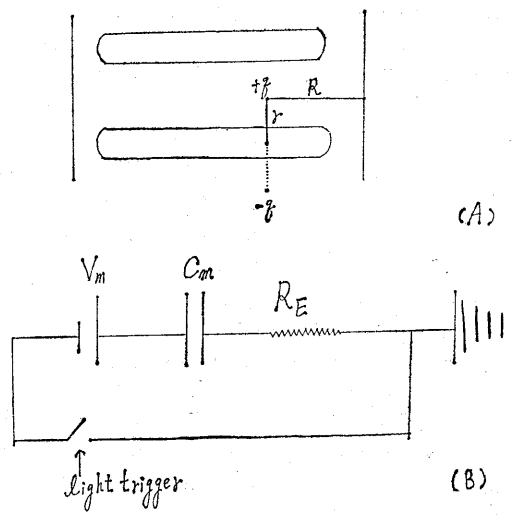

Fig. 2. (A). A diagramatic drawing of a short piece of outer segment. A charge separation will produce a potential at the envelope. (B). A simplified equivalent circuit of the E. R. P. C_m is the envelope capacitor and R_E is the external resistor. V_m is the potential produced by the charge separation as shown in Fig. 2A.

$$V(x)=\frac{q}{4\pi\epsilon R}-\frac{q}{4\pi\epsilon(R^2+4\gamma^2)^{1/2}}$$

The electric field

$$E=\frac{q}{4\pi\epsilon R^2}-\frac{qR}{4\pi\epsilon(R^2+4\gamma^2)^{3/2}}$$

This is the voltage and field from one charge displacement. This charge displacement alone will not produce a voltage at a long distance away, when R becomes large, both V and E will become zero.

The most important of this model is that

the rod envelope is a good capacitor. Outside the envelope we may consider it as a resistor. What the E_{Intra} needs to do is to drive some charge toward and deposite on the envelope, the capacitor. Thus will induce charge from the other side of the envelope. This induced charge may be drawn from anywhere in the world. Therefore we may measure a transient voltage during the movement of these induced charge.

The equivalent circuit may be drawn as Fig. 2B. During the charge separation, the V_m at the envelope is $\sum_i \left(\frac{q_i}{4\pi\epsilon R_i} - \frac{q_i}{4\pi\epsilon(R_i^2+4\gamma^2)^{1/2}} \right)$, i is summed over all the charges each with charge q_i and at distance R_i from the rod envelope.

For one charge $q_i \simeq e$ substitutes $\epsilon \simeq \epsilon_o$. When $R_i \simeq 100$Å, and $\gamma = 20$Å we have $V_m \simeq 10$ *mV*. This is approximate the voltage that can be measured intracellularly and it is also the largest voltage can possibly be measured extracellularly. (Fig. 2B). As long as the charge separation occurs, the change of ionic condition or chemical composition will only change the speed of the appearance of the response as described by Brindley *et al.*[4].

R2 GENERATING MECHANISM

The response of R2 cannot be detected as the temperature approaches 0°C or at pH below 4[3,4]. This response can not be explained by the Meta I to Meta II transition, because at lower pH, the transition should be more complete and should not be terminated. R2 is the dominant component at physiological conditions and it appears immediately prior to the L. R. P. —the neural response indicating that it may be the most important signal for transduction.

This response may be generated by a similar mechanism: separation of charges followed by the spreading of one pole of the dipole as described for R1. However, the mechanism by which the charges are separated and spreading are probably different. Let us examine another example.

If some groups, for example the S^- on the solid surface absorbs some positive charges H^+. The negative charge will remain in the cytoplasm in the form of negative ions. These negative ions will not all pile up parallel to the positive charges. Because of thermal motion, all ions in the electrolyte tend to equalize their concentration. This will produce diffused electrical double layers[13]. This is another type of dipole. The positive pole is on the double membrane disks which carries the sulfhydryl groups while the negative pole is the cytoplasm. After the charge separation, the ions will have a distribution according to Boltzmann *Eq.*

$$\rho_i = \rho_{oi} \exp(-e_i\phi/kT) \tag{1}$$

where ϕ is the potential due to the presence of the positive charges on the membrane. This potential extends into the interior of the electrolyte (cytoplasm). ρ_{oi} is the concentration of the ion of type i with charge e_i at $\phi = 0$, and e_i can be either positive or negative, k is the Botzmann constant and T is the absolute temperature. The charge density is:

$$\rho = \sum_i \rho_i e_i \tag{1'}$$

From one dimentional Poisson's *Eq.*

$$\frac{\partial^2\phi}{\partial x^2} = -(4\pi/\varepsilon)\rho \tag{2}$$

where ε is the dielectric constant of the cytoplasm and x is the distance from the membrane.

Substituting (1′) into (2) we have

$$\frac{\partial^2\phi}{\partial x^2} = -(4\pi/\varepsilon)\sum_i \rho_{oi} \exp(-e_i\phi/kT)e_i \tag{3}$$

For $e_i\phi \ll kT$, the Debye and Hückel's approximation gives

$$\frac{\partial^2\phi}{\partial x^2} = -(4\pi/\varepsilon)\sum_i (\rho_{oi} - \rho_{oi}e_i\phi/kT)e_i \tag{4}$$

For electroneutrality,

$$\sum_i \rho_{oi}e_i = 0 \tag{5}$$

but if the electroneutrality does not hold in

the outer segment, $\sum_i \rho_{oi} e_i = C$. This contant C will give a constant potential shift which may be defined as the baseline. This constant potential shift will not influence the result just as the dark current which is defined as baseline does not have any effect on the measurement of E.R.P.

$$\frac{\partial^2 \phi}{\partial x^2} = \sum_i (4\pi \rho_{oi} e_i^2 / \varepsilon kT)\phi \qquad (6)$$

Let

$$K^2 = (4\pi e^2/\varepsilon kT)\sum \rho_{oi} Z_i^2; \quad e_i = eZ_i$$

We get

$$\phi = V \exp(-Kx) \qquad (7)$$

From the potential at $x=0$, we have

$$V = -(4\pi/\varepsilon K)Q_m \qquad (8)$$

Q_m is the charge absorbed by a unit area of membrane. ϕ is proportional to Q_m which in turn is proportional to the number of rhodpsins bleached. This will explain the linear relationship between the E.R.P. and the number of rhodopsins bleached[2]. When the number of bleached rhodopsin is very large, Q_m will become very large. According to *Eq.* 8, V also seems to become very large. But when $eV \simeq kT$, the electrostatic force will overcome the thermal motion and attract more negative ions to membrane. This will reduce the net Q_m on the members and in turn the V. Therefore there is a saturation value for V; $V < kT/e$. The magnitude of V at saturation is about 25 mV. For intracellular recording, the microelectrode is so large that it can not reach the membrane to measure the V, thus the measurable V_{max} will be a fraction of the V_{max}. For extracellular recording, the saturation value depends on the detailed structure of the outer segment and the place where the measurement is made. (Fig. 2B).

Suppose some charge Q is suddenly absorbed by the membrane, then the ions remaining in the cytoplasm will redistribute themselves to the final state as described by equations (1) and (7). For the transient movement, we will start with Nernst *Eq.* of ionic mobility. $N_i(t, x)$ is the number of mobile ion for type i at the time t and position x. D_i and ρ_i are the diffusion coefficient and density of this ion. Total mobile ions are given by the following:

$$N(t, x) = \sum_i N_i(t, x) = \sum_i -D_i\left[\frac{\partial \rho_i}{\partial x} + \frac{e_i \rho_i}{kT}\left(\frac{\partial \phi}{\partial x}\right)\right] \qquad (9)$$

From the equation of continuity

$$\frac{\partial \rho}{\partial t} = -\nabla \vec{J} \qquad (10)$$

$\vec{J} = N_i e_i / A$ for a one dimensional uniform flow, where A is the area of the flow. Thus

$$\frac{\partial \rho}{\partial t} = -(1/A)\left(\frac{\partial \sum_i N_i e_i}{\partial x}\right) \qquad (11)$$

Substituting (9) into (11)

$$\frac{\partial \rho}{\partial t} = \sum_i \frac{D_i}{A}\left[\frac{\partial^2 \rho_i}{\partial x^2} + \frac{e_i \rho_i}{kT}\frac{\partial^2 \phi}{\partial x^2}\right] e_i \qquad (12)$$

From Poisson's equation

$$\frac{\partial^2 \phi}{\partial x^2} = -\frac{4\pi}{\varepsilon}\rho$$

We have

$$\frac{\partial \rho}{\partial t} = \sum_i \frac{D_i}{A}\left[\frac{\partial^2 \rho_i}{\partial x^2} - \frac{e_i \rho_i}{kT}\frac{4\pi \rho}{\varepsilon}\right] e_i \qquad (13)$$

The movement of ions depends on the diffusion term $\frac{\partial^2 \rho_i}{\partial x^2}$ and the electrostatic force term $\frac{e_i \rho_i}{kT}\frac{4\pi\rho}{\varepsilon}$. When positive charges are suddenly absorbed by the membrane, there is a small volume adjacent to the membrane with thickness δ where negative ions are concentrated. Because of thermal motion and electrostatic force, these negative ions will move away and some positive ions will move into this small volume. After the absorption of ion the diffusion term will only allow the hydrogen ions to enter, because in our case, the trapped ions are the hydrogen ions and only the hydrogen ions has a concentration gradient. The electrostatic force favor any positive ions to enter and any negative ions to exit. Since $\rho = \sum_i \rho_i e_i$, any ion with

larger mobility will flux in or out and change the value of ρ, and this will impede the motion of slow moving ions. The mobility of hydrogen ions is five to ten times that of other ions. Therefore with reference to the diffusion term or to the electrostatic force term, the moving ions are dominated by hydrogen ions. The change of ρ is also dominated by the change of hydrogen ion concentration as described by $\rho \simeq (\rho_h - \rho_{oh})e$, where ρ_h: hydrogen ion concentration and ρ_{oh}: hydrogen ion concentration before photo-excitation. Substitute $\rho \simeq (\rho_h - \rho_{oh})e$ into *Eq.* (13), we have

$$\frac{\partial \rho_h}{\partial t} = \frac{D_h}{A}\left[\frac{\partial^2 \rho_h}{\partial x^2} - \frac{e^2 \rho_h}{kT}\,\frac{4\pi}{\varepsilon}(\rho_h - \rho_{oh})\right] \quad (14)$$

The boundary conditions are $\rho_h(o, x) = 0$ at $x < \delta$, $\rho_h(o, x) = \rho_{oh}$ at $x > \delta$, where δ is the initial thickness of the double layers, and

$$\rho_h(\infty, x) = \rho_{oh} \exp\left[eV \exp\left(-Kx\right)/kT\right] \quad (15)$$

The double layers extends slowly from a very small thickness according to *Eq.* (14). This electrical double layers cannot extend a great distance from the membrane. The final situation is shown in *Eq.* (15) and the equilibrium potential is

$$\phi = V \exp\left(-K\,x\right) \quad (7)$$

This potential extends into the cytoplasm, but the magnitude decreases to $1/e$ at $x = 1/K$. At pH=7, with $[H^+] = 6.02 \times 10^{13}/cm^3$ we have $1/K \simeq 10^4$Å, thus the diffused double layers will extend about 10^4Å. At pH=3, with $[H^+] = 6 \times 10^{17}/cm^3$, we have $1/K \simeq 10^2$Å, the diffused double layers can only be extended to about 100Å. Therefore at pH=3, it would be difficult for the diffused double layers to extend from the double membrane disks to the envelope and thus can not be measured extracellulary.

Similarly, the cone envelope is directly connected to the double membrane disks while the rod envelope is separated a few hundred Å from the double membrane disks. Therefore the spreading of charges will reach the cone envelope more easily and deposit more charge on it, resulting in E. R. P_{extra} to be much larger in cone than in rod.

Origin of the R1 and R2

R1 is probably a result of a direct charge separation. The separation should be of considerable distance. One of the possible candidates is the 11-cis to all-trans isomerization, because it occurrs early in the photo-excitation and possibly represents the largest movement in all of the photo-excitation processes. It can not be stopped by freezing to 0°C, however it can be slowed down by freezing to -30°C. Only when the solution reachs -140°C this isomerization can be stopped[15]. It would be interesting to study the temperature effect on the R1 and compare it with the temperature effect on the 11-cis to all-trans isomerization.

The origin of R2 is probably due to the absorption of ions and then movement of ions. The ion absorption and movement are stopped at temperature near 0°C. This will explain the temperature effect on R2[3,4]. The pH dependeuce strongly suggests that the charges absorbed are H^+. The sulfhydryl groups are the most probable sites on the membrane to absorb the hydrogen ions[14]. Direct evidence has been obtained by Brindley *et al.*[8] They treated the retina with N-ethyl maleimide, a thiol blocking agent, and observed that the magnitude of R2 has been greatly reduced at neutral pH and room temperature.

Relationship between E. R. P. and L. R. P.

Toyoda *et al.*[6] demonstrated that E. R. P. is not the direct cause for L. R. P. However, this does not rule out the possibility that the charged particles that cause the E. R. P. are the transmitters for the L. R. P.. Although Toyoda[6] *et al.* proved that the coupling is not a electrical one, it does not rule out the possibility that the coupling is a chemical one comparable to other neurotransmitter mechanisms. In addition to Ca^{++}, another candidate worthy of investigation is the OH^- ion which remains in the cytoplasm and migrates toward the envelope

after photo-excitation.

Discussion

It is clear that the model of charge separation and subsequent charge spreading is sufficient to explain the temperature dependence and the conductance dependence of R1, as well as the pH and temperature dependence of R2 and the linear relationship to the amount of bleached rhodopsins. Both R1 and R2 should be much easier to detect extracellularly for cones because the charges need to extend a shorter distance to reach the envelope than for the rods. This model also makes use of the finding of Murakami *et al.*[7] that the envelope is a capacitor good enough to allow for a long distance travel of the response extracellularly. As to the spatial distribution of the potential and current, the proposed modal is compatible with the model proposed by Rüppel and Hagins[8]. There is a simple way to test the two models. The experiment of Rüppel and Hagins must be repeated by measuring the spatial distribution in a three dimensional way instead of along the rod outer segment, and the spatial distribution should be measured at different distance from the rod envelope as well as along the rod to determine whether current distribution is more like a "source" with a "sink" in the rod, as Rüppel and Hagins suggested, or like a single "source" for R1 and like a single "sink" for R2 at the outer segment. According to Rüppel and Hagins, intracellular recording of E.R.P. should detect the potential change polarity with respect to E.R.P. extracellular. However, this had not been observed by Murakami *et al.*[7] Because the "sink" and "source" in the model of Rüppel and Hagins are both inside the rod, the E.R.P. will probably be difficult to measure at a far distance from the retina, for example in the cornea of the eye[2].

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脊椎動物視網膜接受體早期電位產生之動作原理

王 唯 工

一個正負電分離後，一電極擴散開的模型，可以解釋視網膜接受體之早期電位。R1 信號係由正負電直接分離產生。R2 信號是由離子的吸附及後隨之離子移動而產生，由此產生一擴散的雙電層。桿細胞之細胞封套是一個高電容器，因爲這一良好電容性質，由正負電分離所產生之電場，只須將電充於電容器上，而封套外邊所引起之感應電就能產生一瞬息電位，此電位可在遠處量得。所吸附之離子可能爲氫離子，氫氧離子亦推斷，可能爲接受體後期電位的信號物質。