Ca\textsuperscript{2+} Fluxes and Channel Regulation in Rods of the Albino Rat

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ABSTRACT By use of microelectrodes, changes in the receptor current and the Ca\textsuperscript{2+} concentration were measured in the rod layer of the rat retina after stimulation by flashes or steady light. Thereby light induced Ca\textsuperscript{2+} sources, and sinks along a rod were determined in dependence of time. Thus, the Ca\textsuperscript{2+} fluxes across the plasma membrane of a mammalian rod could be studied in detail. By light stimulation, Ca\textsuperscript{2+} sources are evoked along the outer segment only. Immediately after a saturating flash, a maximum of Ca\textsuperscript{2+} efflux is observed which decays exponentially with $\tau = 0.3 \text{ s at 37}^\circ\text{C (4.2 s at 23}^\circ\text{C). During regeneration of the dark current, the outer segment acts as a Ca\textsuperscript{2+} sink, indicating a restoration of the Ca\textsuperscript{2+}-depleted outer segment. These findings agree with earlier reports on amphibian rods. Further experiments showed that the peak Ca\textsuperscript{2+} efflux and $\tau$ are temperature dependent. The peak amplitude also depends on the external Ca\textsuperscript{2+} concentration. In contrast to the reports on amphibian rods, only a part of the Ca\textsuperscript{2+} ions extruded from the outer segment is directly restored. Surprisingly, during steady light the Ca\textsuperscript{2+} efflux approaches a permanent residual value. Therefore, in course of a photoresponse, Ca\textsuperscript{2+} must be liberated irreversibly from internal Ca\textsuperscript{2+} stores. There is certain evidence that the inner segment acts as a Ca\textsuperscript{2+} store. Our results show that the Ca\textsuperscript{2+} fraction of the ions carrying the dark current is proportional to the extracellular Ca\textsuperscript{2+} concentration. This indicates that the Ca\textsuperscript{2+} permeability of the plasma membrane of the rod outer segment is independent of the Ca\textsuperscript{2+} concentration. Key words: rat rod photoreceptors • Ca\textsuperscript{2+}-sensitive microelectrodes • light-induced Ca\textsuperscript{2+} fluxes • Ca\textsuperscript{2+} source function • intracellular Ca\textsuperscript{2+} concentration

INTRODUCTION

In the dark, an Na\textsuperscript{+} current circulates between the inner and outer segment of the rod photoreceptor (Hagins et al., 1970; Penn and Hagins, 1972). This dark current is generated in the inner segment by means of an Na\textsuperscript{+}/K\textsuperscript{+} ATPase. From the inner segment the dark current flows extracellularly to the outer segment, entering the rod again via cGMP-dependent ion channels. Light absorption by rhodopsin initiates a reduction of the dark current: Activated rhodopsin (R*)\textsuperscript{1} causes a G-protein-mediated stimulation of a cGMP-specific phosphodiesterase (PDE) leading to an enhanced hydrolysis of cGMP. As a consequence, the free cGMP concentration in the outer segment falls. Thus, the cGMP-dependent ion channels close so that the Na\textsuperscript{+} influx into the outer segment decreases (for review see Pugh and Lamb, 1990).

In addition to the Na\textsuperscript{+} influx a remarkable flow of Ca\textsuperscript{2+} ions into the outer segment is observed through the light-regulated ion channels (Hodgkin et al., 1985; Nakatani and Yau, 1988). In the dark, the Ca\textsuperscript{2+} influx is balanced by a Ca\textsuperscript{2+} efflux from the outer segment via an Na\textsuperscript{+}/K\textsuperscript{+} - Ca\textsuperscript{2+} exchanger (Yau and Nakatani, 1985; Cervetto et al., 1989; Friedel et al., 1991). A light-induced closure of the cGMP-dependent ion channels also reduces the Ca\textsuperscript{2+} influx into the outer segment. Thus, after light stimulation, a net Ca\textsuperscript{2+} efflux remains so that the Ca\textsuperscript{2+} concentration is increased in the extracellular medium (Yoshikami et al., 1980; Gold, 1986; Cieslik and Rüppel, 1988) but reduced in the outer segment (Yau and Nakatani, 1985; Gold, 1986; Miller and Korenbrot, 1987; Ratto et al., 1988).

In amphibian rods, the reduction of the Ca\textsuperscript{2+} concentration in the outer segment after a flash of light accelerates the restoration of the dropped cGMP concentration to the dark level (Koch and Stryer, 1988; Yau and Nakatani, 1988; Matthews, 1991) and causes an adaptation to steady background light (Matthews et al., 1988). Both effects can be explained by a stimulated activity of a guanylate cyclase (GC) that resynthesizes cGMP (Koch and Stryer, 1988). Since the GC is known to be inhibited by Ca\textsuperscript{2+} ions, the cGMP synthesis becomes activated if the Ca\textsuperscript{2+} concentration in the outer

\textsuperscript{1}Abbreviations used in this paper: GC, guanylate cyclase; LED, light-emitting diode; PDE, phosphodiesterase; R/R*, rhodopsin/activated rhodopsin; ros, rod outer segment.
segment is reduced. However, the action of Ca$^{2+}$ on the transduction pathway seems to be more complex: Ca$^{2+}$ ions are also reported to effect the rhodopsin deactivation (Wagner et al., 1989; Knopp and Rüppel, 1993; Kawamura, 1993; Knopp, 1994), to reduce the R*/PDE gain (Pepperberg et al., 1994; Lagano and Baylor, 1994), and to interact with the cGMP-dependent ion channels from the cytoplasmatic side (Hsu and Molday, 1993).

Because of the various interactions of Ca$^{2+}$, it is important to know how light alters the Ca$^{2+}$ concentration and the Ca$^{2+}$ fluxes in the outer segment. Under physiological conditions, the Ca$^{2+}$ concentration in the outer segment can be directly measured solely during completely interrupted dark current (Ratto et al., 1988). Until now the Ca$^{2+}$ efflux was mainly determined by the measurement of the Ca$^{2+}$ exchange current in amphibian rods using suction electrodes (Hodgkin et al., 1987; Yau and Nakatani, 1988; Yau and Nakatani, 1985; Nakatani and Yau, 1988). With mammalian rods, only one detailed study about the Ca$^{2+}$ efflux was performed by these suction electrodes (Tamura et al., 1991). As the Ca$^{2+}$ exchange current is obtained during complete interruption of the dark current only, the method does not allow for the observation of the Ca$^{2+}$ exchange current after stimulation by dim flashes and during the time period of the dark current regeneration.

In a few studies of amphibian rods, Ca$^{2+}$-selective electrodes were used to derive the Ca$^{2+}$ fluxes from measurements of the extracellular Ca$^{2+}$ concentration (Gold, 1986; Miller and Korenbrot, 1987). However, since differences between amphibian and mammalian rods are suggested (Pugh and Altman, 1988), the results from amphibian rods may not be assigned to mammalian rods. Only one unique determination of the Ca$^{2+}$ efflux from mammalian rods was performed by use of Ca$^{2+}$-selective electrodes (Yoshikami et al., 1980). However, in this study only one asymmetric electrode was used. Moreover, a disadvantage of all these methods used until now is that a uniform distribution of Ca$^{2+}$ sources along the outer segment had to be assumed.

We here report on a special method to determine the Ca$^{2+}$ fluxes through the rod plasma membrane from simultaneous measurements of the dark current and the Ca$^{2+}$ concentration in the extracellular space of the photoreceptor layer. From the measured data, the net Ca$^{2+}$ efflux is obtained by applying a specific, one-dimensional diffusion equation. In contrast to other approaches, the new method enables the Ca$^{2+}$ fluxes to be determined even at distinct sites along the tiny mammalian rods. The method was used to study Ca$^{2+}$ fluxes in rat rods during a photoresponse and to determine the time course of the free Ca$^{2+}$ concentration in the rod outer segment. Our results mainly agree with earlier findings obtained from amphibian rods. It is shown that light causes the outer segment to be the main Ca$^{2+}$ source. However, unexpectedly, only a part of the Ca$^{2+}$ extruded from the outer segment during a photoresponse is restored, and a permanent Ca$^{2+}$ efflux from the outer segment during steady light is obtained. The results are discussed in terms of Ca$^{2+}$ fluxes that may be present in the rod.

MATERIALS AND METHODS

Materials

Albino Wistar rats were obtained from Schering AG (Berlin, Germany). The Ca$^{2+}$-selective liquid membrane (Ca-Cocktail A), carbon tetrachloride (CCl$_4$), tetrahydrofuran, and trimethylchlorosilane (Me$_3$SiCl) were purchased from Fluka (Neu Ulm, Germany).

Incubation Medium and Retinal Preparation

Ringer's solution was prepared after Hagins et al. (1970) consisting of 130 mM NaCl, 2.2 mM KCl, 0.18 mM MgCl$_2$·6 H$_2$O, 11 mM glucose, 1.3 mM KH$_2$PO$_4$, 5.4 mM Na$_2$HPO$_4$, and 10 mM HEPES. The solution was titrated to pH 7.4 by NaOH. The final Ca$^{2+}$ concentration was adjusted by adding appropriate amounts of a CaCl$_2$ stock solution.

Albino rats were kept in complete darkness for 2 h or more before they were killed by peritoneal injection of 2 ml of the Na$^+$ pentobarbiturate (Nembutal®, Ceva AG, Bad Segeberg, Germany). After cardiac arrest, the eyes were enucleated and the bulbus was cut in the meridian into two halves. The lower eye cup containing the retina was transferred into Ringer's solution. After ~10 min, the retina was gently removed from the pigment epithelium and was stored in Ringer's solution at room temperature in darkness. For recordings, pieces of ~1 mm$^2$ of the retina were placed receptor side up on a cellulose acetate filter (SM 11104; Satorius, Göttingen, Germany) and transferred into the recording chamber. The preparation was carried out under dim red light.

Recording Chamber and Photostimulation

The recording chamber (Fig. 1) consisted of a glass cuvette filled with Ringer's solution and grounded by an Ag/AgCl wire. The cuvette was embedded in a black anodized aluminium block, which was thermostated by a Peltier element. The perfusion of the recording chamber with Ringer's solution was driven by gravity. The perfusate was collected by suction.

The illumination of the piece of retina within the recording chamber was achieved from below the chamber using flashes produced by a pulsed LED (Rüppel et al., 1978) or steady light via a splitted waveguide. The light intensity was attenuated by neutral density filters (No. 96; Eastman Kodak Co., Rochester, NY).

Electrode Preparation and Recording Assembly

From above the recording chamber, a double-barreled recording microelectrode (1, 2) was moved into the retina between the photoreceptor cells (cf. Fig. 1). This was performed by a stepper motor (AM2 M2; Bachoer, Reutlingen, Germany) with a
FIGURE 1. Schematic representation of the measuring device with flow chamber and assembly of double- and single-barreled electrodes. By the Ca\(^{2+}\)-sensitive barrel (1), the Ca\(^{2+}\) concentration is detected, whereas the voltage-sensitive barrel (2) measures simultaneously a voltage produced by the dark current in the extracellular space between the rods. The reference electrode (3) is positioned at the rod tips of the retinal layer. The arrangement of difference amplifiers directly yields output voltages that are dependent on changes of the Ca\(^{2+}\) concentration (Ca\(^{2+}\) signal) and the extracellular potential (photosignal).

FIGURE 2. Schematic representation of the free extracellular space between the rods. (a) Axial view showing the change in cross-section area versus penetration depth \(z\). (b) Cross-section of a rod array at the tip of outer segment \((z = 0)\). \(A_o, A, A'\): cross-section areas at different penetration depths \(0, z, z'\).
rod dark current, had to be considered. This was especially important if the retina was successively stimulated by steady light. In this case, several minutes of intermission were allowed in between to ensure a complete regeneration of the retina back to the dark state. Thus, the total measuring time added up to at least 30 min. With respect to retinal stability during this measuring period, the results of earlier long-time studies of the isolated rat retina in the course of electrode insertion could be used for reference. These studies revealed a half-life of the retina between 50 and 500 min (Kuhl et al., 1995). With respect to possible retinal damage during electrode insertion, however, thorough bracketing measurements performed by Hagins et al. (1970) show that there is no hysteresis in the dark voltage trace between insertion and withdrawal of the electrode in a rat retina. To prove whether this observation applies also to the recording of the Ca\textsuperscript{2+} concentration, particular control measurements were performed. One example is shown in Fig. 5. The maximum amplitude value as well as the half-rise time of Ca\textsuperscript{2+} signals are plotted versus the penetration depth \(z\). The signals with numbers \(N = 1-13\) were recorded during the insertion of the electrode from above the retina \((z = -48 \mu m)\) deeply down into the nuclear layer \((z = -56 \mu m)\), whereas the Ca\textsuperscript{2+} signal \(N = 14\) was evoked during the withdrawal at \(z = 8 \mu m\), i.e., at the most sensitive location of maximum amplitude. The amplitude value and the half-rise time of the control signal \((N = 14)\) fit perfectly to the set of signal data measured at neighboring positions. This would not be the case if retinal damage caused by the electrode insertion as well as a significant run-down of the receptor current had taken place during this experiment. Therefore, the control measurement shows that the procedure of electrode insertion yields reversible results.

**Theory**

**Ca\textsuperscript{2+} ion diffusion in the extracellular space.** Light-induced changes of Ca\textsuperscript{2+} concentration are measured in the extracellular space of the retinal rod layer (see Fig. 2). These concentration changes result from Ca\textsuperscript{2+} transport processes across the plasma membrane of the rods and diffusion processes outside the rods. The extracellular space is defined by the lateral arrangement of the closely packed rods, which on the average can be represented by a quadratic lattice. Thus, it is evident that the outer space in between four rods shows an axial symmetry with respect to a longitudinal axis (see cross-section in Fig. 2). The Ca\textsuperscript{2+} transport processes in this outer space are described by the diffusion equation

\[
\frac{\partial C}{\partial t} + \text{div} j = q(x,y,z,t)
\]

which in cylindrical coordinates is given by

\[
\frac{\partial C}{\partial t} - D \left( \frac{\partial^2 C}{\partial z^2} + \frac{1}{r^2} \frac{\partial^2 C}{\partial \phi^2} + \frac{1}{r} \frac{\partial \phi C}{\partial \phi} \right) = q(z,r,\Phi,t),
\]

where \(z\) denotes the longitudinal axis, \(\rho\) is the direction of electrode penetration, \(\rho\) is the Ca\textsuperscript{2+} concentration in the extracellular space, \(j = -D \cdot \text{grad} \rho\), the Ca\textsuperscript{2+} flux density, \(D\) is the diffusion constant for Ca\textsuperscript{2+} ions, and \(q(z,r,\Phi,t)\) is the source function for which the time-dependent sources and sinks are localized at the boundaries of the extracellular space, i.e., the rod surfaces.

As the radial distances between the rods are much smaller than the longitudinal pathway for the Ca\textsuperscript{2+} ion flux, the equilibration of concentration gradients proceeds much faster in the radial than the axial direction. Thus, in a first approximation, for any cross-section area \(A(z)\), the radial concentration gradient \((\partial \rho / \partial r)\), as well as the circumferential gradient \((\partial \rho / \partial \phi)\), should be zero so that the two lateral terms in Eq. 1 could be neglected. Even under the assumption of diminishing lateral gradients, however, a radial flow of Ca\textsuperscript{2+} ions must be considered if the cross-section area alters in axial direction, i.e., \(\partial A/\partial z \neq 0\). This radial flow results in a considerable contribution to the source function, which has to be allowed for if the net Ca\textsuperscript{2+} flux across the rod plasma membrane is being determined. In fact, the actual source function can be derived from the divergence of the axial Ca\textsuperscript{2+} ion flux if changes in the cross-section area are taken into account: For an axial flow of Ca\textsuperscript{2+} ions

\[
\frac{\partial C}{\partial t} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) = \frac{\partial}{\partial r} \left( r j \right)
\]

The graphs in Fig. 3 show the relationship between the maximum amplitude (\(D\)) and half-rise time \(t_{1/2}\) (\(\bigcirc\)) of Ca\textsuperscript{2+} signals measured at different positions \(z = -48 \mu m\) to +56 \mu m while inserting the double-barreled electrode into the rod layer of a rat retina (signal No. 1-13) The rod tips were positioned at \(z = 0\). The retina was stimulated by steady light of saturating intensity of 10 s duration. The time period between each singular measurement was 3 min. Regular step width was 8 \mu m. The signal at position \(z = 8 \mu m\) (No. 14) was recorded after the electrode was withdrawn from the inner nuclear layer. Amplitude and rise time of signal No. 14 (\(\bigcirc\) and \(\bigotimes\)) fit well with the Ca\textsuperscript{2+} signal data obtained during electrode insertion at neighboring positions.
\[ j(z) = -D \frac{\partial c}{\partial z}(z), \]

the divergence within a space interval between \( z \) and \( z' = z + \Delta z \) and boundary areas \( A \) and \( A' = A + \Delta A \) is approximated by

\[
\text{div} j(z) = \frac{A' - j(z') - A - j(z)}{\Delta V} A' z
\]

\[
= - \frac{A' \left( \frac{\partial c}{\partial z}(z + \Delta z) - \frac{\partial c}{\partial z}(z) \right)}{\Delta z}
\]

\[
= -D \frac{\Delta A}{A} \frac{\partial c}{\partial z}(z + \Delta z) - D \frac{\Delta A}{A} \frac{\partial c}{\partial z}(z),
\]

where \( \Delta V = \bar{A} \cdot \Delta z \) \( \bar{A} = (A + A')/2 \) is the average volume (cf. Fig. 2). In the limit \( \Delta z \to 0 \) \( (\bar{A} \to A) \), the divergence follows as

\[
\text{div} j(z) = -D \left( A' \frac{c(z + \Delta z) - c(z)}{\Delta z} - A \frac{c(z) - c(z - \Delta z)}{\Delta z} \right).
\]

According to Eq. 1, the function \( q(z,t) \) is approximated by

\[
q(z,t) = \frac{\partial c}{\partial t} - \frac{2D}{\Delta z_0 (A' + A)} \left( A' \frac{c(z + \Delta z) - c(z)}{\Delta z_0} - A \frac{c(z) - c(z - \Delta z)}{\Delta z_0} \right). \quad (5)
\]

Eq. 5 is used to calculate the source function from the measured data.

**Determination of cross-section \( A(z) \) in the extracellular space.** The cross-section area was estimated as follows: At the tip of the outer segment at \( z = 0 \), the area \( A(0) \) was estimated by assuming an arrangement of rods within a quadratic lattice. If the rods occupy the lattice points, the lattice constant is \( 2r \) \( (r = \text{radius of outer segment}) \) and the cross-section area of the free space between the rods is

\[
A(0) = (4 - \pi) r^2.
\]

Nearly the same value \( (\text{exactly } 0.74 \mu m^2) \) was measured by us for the free interstitial space from a transmission electron micrograph taken by Hagins (1973, unpublished) showing a lateral cut through the rod outer segment layer of a rat retina.

\[
\text{Table I}
\]

| \( z/\mu m \) | 0 | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 |
|-----------------|---|---|----|----|----|----|----|----|----|----|----|
| \( A(z)/A(0) \) | 1 | 0.99 | 0.96 | 0.84 | 0.60 | 0.33 | 0.17 | 0.15 | 0.14 | 0.13 | 0.13 |

Table I: Relative Cross-Section Area of Free Extracellular Space

(a) The relative cross-section area of the free extracellular space in dependence of the penetration depth \( z \) was derived from the longitudinal resistance \( R_z \) per unit length as measured by Hagins et al. (1970) within the isolated life rat retina. The longitudinal resistance is inversely proportional to \( A(z) \); thus \( A(z) = \frac{R_z}{R_z} \cdot A(0) \). (B) For data smoothing, an algorithm reported by Allen and Tildesley (1987) was used. \( A_n \) denotes data values before, \( A_n' \) after applying a smoothing operation. Eqs. 1 and 2 are applied to obtain the first and second, whereas Eq. 4 and 5 are applied to yield the last two of the smoothed values. Eq. 3 was used to smooth the other values of the data set. The smoothing operation was applied alternatingly three times each in space and time. The step width was \( \Delta z = 8 \text{ mm} \) and \( \Delta t = 30 \text{ ms} \).
The data of the cross-section area $A(z)$ is derived from the longitudinal resistance per unit length

$$R_z = \frac{dR}{dz}$$

of the extracellular space as measured by Hagins (Hagins et al., 1970): The longitudinal resistance is inversely proportional to $A(z)$ so that

$$A(z) = \frac{[R_r(z)/R_n(z)] \cdot A(o)}.$$

**Calculation of net transmembrane $Ca^{2+}$ fluxes.** The net efflux of $Ca^{2+}$ ions from the rod [l/s] into the adjacent infinitesimal volume element $dV = A(z)dz$ is derived from the source function $q(z,t)$ [M/s] by

$$dQ(z,t) = q(z,t) \cdot N_A^{-1} \cdot A(z) \cdot dz \text{ or per unit length as}$$

$$dQ(z,t) = q(z,t) \cdot A(z). \quad (6)$$

where $N_A$ is Avogadro’s constant.

Consequently, the total net $Ca^{2+}$ efflux $Q(z,t)$ between the position $z_i$ and $z$ is obtained by the axial integral

$$Q(z,t) = \int_{z_i}^z q(z,t) \cdot A(z) \cdot N_A^{-1} \cdot dz.$$

Thus, the net $Ca^{2+}$ outflow $Q_{out}(t)$ from the whole outer segment, i.e., between $z = 0$ and $z = 25 \mu m$ is given by

$$Q_{out}(t) = \int_{z_i}^{z_f} q(z,t) \cdot A(z) \cdot N_A^{-1} \cdot dz. \quad (7)$$

The amount of $Ca^{2+}$ ions that is pumped out of the rod plasma membrane at the position $z$ into the space volume element $dV = A(z)dz$ between $t = 0$ (time of light on) and the time $t$ after light onset is given by

$$\Delta Ca_z = \int_0^t Q(z,t) \cdot dt. \quad (8)$$

Finally, the total amount of $Ca^{2+}$ extruded from the whole outer segment at the time $t$ after light onset is

$$\Delta Ca_{out} = \int_0^t Q_{out}(t) \cdot dt. \quad (9)$$

In practice, the integral $Q_{out}(t)$ of Eq. 6 can be approximated by a sum

$$Q_{out} = \sum_{i=1}^3 \Delta Q(z_i) = \sum_{i=1}^3 (qA)_{z_i} \cdot N_A^{-1} \cdot \Delta z_i$$

$$= \left( (qA)_{z_0} + (qA)_{z_1} + (qA)_{z_2} \right) \cdot N_A^{-1} \cdot \Delta z_0 \quad (10)$$

where $A_{z_i}$ is the mean value of the cross-section area between $z_i$ and $z_{i+1}$.

**Data smoothing.** For the numerical calculation of the source function according to Eq. 4, the data of the $Ca^{2+}$ measurements were smoothed by use of an algorithm reported by Allen and Tilkesley (1987), which is given in Table I. This algorithm was originally developed for one independent variable only. For this calculation, however, the data smoothing procedure was applied $n = 3$ times alternatingly in time and penetration depth $z$. The sample interval was $\Delta z = 8 \mu m$ in $z$ and $\Delta t = 30 m s$ in $t$. The smoothing operation was extended over two intervals on each side of the sample point (see Table I). For each run, a limitation in the signal bandwidth in space or in time occurs that can be approximated by a linear integration step over $m = 3$ sample intervals. For a single application of the smoothing algorithm according to Shannon’s theorem, the bandwidth of the signal presentation is limited to $\Delta f = (2 \cdot m \cdot \Delta z)^{-1}$ in the $t$-axis and to $\Delta f' = (2 \cdot m \cdot \Delta z')^{-1}$ in the $z$-axis ($f$ = “time” frequency, $f'$ = “space” frequency, Rüppel, 1983). An $n$-times repetition of this procedure corresponds to a series connection of $n$ equal band path filters with a time or space constant of $\tau_n = 4\Delta t$ and $\lambda_n = \Delta z$, respectively. Finally, an alternating application of the smoothing procedure in time and space yields the product of both bandwidth reduction factors. Thus, in this particular case,

$$\Delta f = (2^{2n} - 1) \cdot m^{-2} \cdot \Delta t_0 = 4 Hz,$$

which corresponds to a rise time of 0.9 s for $\Delta t_0 = 13 Hz$. For $n = 3$, $m = 3$.

**RESULTS**

**Changes of Extracellular $Ca^{2+}$ Concentration after Flashes of Light**

Flash-induced changes of the extracellular $Ca^{2+}$ concentration were measured in the extracellular space. Fig. 4 represents $Ca^{2+}$ changes $\Delta [Ca_o]$ recorded at different retinal depths $z$ in dependence of the time $t$ after a saturating flash that completely interrupted the dark current for $\sim 1.5 s$. The $Ca^{2+}$ signals are plotted in a three-dimensional $\Delta [Ca_o](z,t)$ representation and are shown for four different values of the extracellular $Ca^{2+}$ concentration: (a) 0.1, (b) 0.2, (c) 0.5, and (d) 1 mM. First of all, it is conspicuous that the time courses of the $Ca^{2+}$ concentration changes $\Delta [Ca_o]$ depend on the penetration depth $z$ of the electrode. Near the outer segment ($z = 0$–25 $\mu m$), after flash stimulation, the $Ca^{2+}$ concentration increases with maximum rate and maximum amplitude at all four $Ca^{2+}$ concentrations in the Ringer’s solution (see Fig. 4 a–d). The amplitude of the $Ca^{2+}$ signal depends on the extracellular $Ca^{2+}$ concentration. With the same flash strength, a maximum amplitude of 5 and 10 $\mu M$ is produced if Ringer’s solution contains 0.1 and 1 mM $Ca^{2+}$, respectively. The time course of the $Ca^{2+}$ signals measured near the outer segment is independent of the $Ca^{2+}$ concentration in the Ringer’s solution. These observations suggest, first, that the outer segment acts as the main $Ca^{2+}$ source for the light-induced increase of the extracellular $Ca^{2+}$ concentration, and second, that the rate of the flash-induced $Ca^{2+}$ release from the outer segment rises with the external $Ca^{2+}$ concentration.
the center of the outer segment and $D$ is the diffusion coefficient of Ca$^{2+}$ in Ringer's solution ($D = 1,000 \ \mu m^2/s$, Yoshikami et al., 1980; Rüppel and Cieslik, 1988). Therefore, the elevation of the Ca$^{2+}$ concentration above the retina can be attributed to Ca$^{2+}$ ions that diffuse into the Ringer's solution after being released from the outer segments. This again proves the outer segment to represent the main Ca$^{2+}$ source after flash illumination.

After passing a maximum, the elevated Ca$^{2+}$ concentration starts to return to the dark level again. The most rapid decline is observed near the outer segments. In most of the experiments, the Ca$^{2+}$ concentration between the outer segments transiently falls even below the dark level (see also Fig. 5 b). This indicates that, at the end of a photoresponse, the function of the outer segment reverses from the main Ca$^{2+}$ source to the main Ca$^{2+}$ sink.

Occasionally, at the synapses ($z = 60 \ \mu m$) a strong and rapid flash-induced increase of the extracellular Ca$^{2+}$ concentration was observed. As a rule, however, it was difficult to investigate the origin of this phenomenon. For this purpose a very stable retina was necessary. The retina from which the Ca$^{2+}$ signals shown in Fig. 4 were derived was such a stable one. From this particular retina, Ca$^{2+}$ signals could be repeatedly recorded down to the synaptic region. Furthermore, the retina did not show a remarkable run-down during data collection and solution exchange. In this case the Ca$^{2+}$ increase near the synapse was strong and rapid and depended considerably on the external Ca$^{2+}$ concentration. At $[Ca]_o = 1,000 \ \mu M$, the Ca$^{2+}$ increase at the synapses even exceeded that measured at the outer segments (see Fig. 4 d), but it was much more attenuated at lower Ca$^{2+}$ levels (see Fig. 4, a–c). However, with regard to Ca$^{2+}$ fluxes, this increase should not be overestimated. The intercellular space in the synaptic region is very small, so that even small Ca$^{2+}$ fluxes may cause a dramatic change of the Ca$^{2+}$ concentration. As shown in Fig. 4, a–d, there is a reduced and delayed increase of the extracellular Ca$^{2+}$ concentration between the outer segment and the synaptic region. Therefore, the increased Ca$^{2+}$ concentration near the synapse is not due to a diffusion of Ca$^{2+}$ ions from the outer segment to the synapse. The origin of the increasing extracellular Ca$^{2+}$ concentration at the synapses is still unclear. This difficulty is mainly due to the limited number of stable retinas available. Thus, the problem of Ca$^{2+}$ fluxes in the synaptic region could not be studied sufficiently and in detail.
Photosignals and Ca\(^{2+}\) Signals Recorded in the Outer Segment Region at Different Flash Intensities

The dark current of a rod is measured as a potential difference between the voltage-sensitive barrel (Fig. 1, 2) of the measuring electrode and the reference electrode (Fig. 1, 3), i.e., across a resistance in the interstitial space. The circulating current of the rod passes the plasma membrane of the outer segment via light-sensitive membrane channels that regulate the current. A light-induced, transient closure of the channels decreases the membrane conductance so that the rod current is reduced. The current change, however, causes a drop of the potential difference between the monitoring electrodes and thus produces the photosignal (Penn and Hagins, 1969; Hagins et al., 1970; cf. Kuhls et al., 1995). Therefore, as a rule, the time course of the photosignal should be the same as that of the conductance change, and this should be the case independent of the electrode position. This is supported by the fact that, in contrast to the Ca\(^{2+}\) signals (see above), the time course of the photosignal does not depend on the penetration depth \(z\). This was measured by Lamb et al. (1981) along the outer segment of detached rods. In fact, in this study a photosignal could be measured with high accuracy, i.e., with sufficient signal-to-noise ratio only beyond the outer segment region. That means, even in the case that in the intact retina the relative time course of current inflow is not the same at all positions \(z\) along the outer segment, the photosignal should represent the mean time course of the conductance changes in the outer segment membrane. Hence, the measured photosignal in the inner segment area was used to present the time course of the conductance and, correspondingly, the permeability for Na\(^{+}\) and Ca\(^{2+}\) ions.

In principle, the space dependence and the time course of the outer membrane conductance can be derived also from the extracellular potential \(V\). Measured by the amplitudes of saturated photosignals at different penetration depths, this extracellular potential \(V(z)\) allows for the calculation of the sources and sinks of the receptor current. Such an analysis of receptor currents was performed before by Penn and Hagins (1969) on the basis of photosignal measurements. As the potential gradient is due to other ion fluxes than Na\(^{+}\) alone, this procedure is subject to considerable error (Cieslik and Rüppel, unpublished results). Therefore, in this study it seemed to be more convenient and reliable to take the mean time course of the membrane current directly from photosignals.

Typical photosignals evoked by flashes of increasing strengths are shown in Fig. 5 a. As already shown in previous studies (Hagins et al., 1970; Penn and Hagins, 1972; Rüppel and Cieslik, 1989), weak flashes of up to 250 v/ros produce photosignals with distinct peak amplitudes that increase with flash strength by a hyperbolic function. More intense flashes saturate the photosignal. These saturating photosignals show a plateau
phase during which the dark current is completely suppressed. Increasing strength of the saturating flashes prolongs the plateau phase of the photosignal.

In Fig. 5 b, Ca$^{2+}$ signals that were recorded simultaneously with the photosignals shown in Fig. 5 a by means of the Ca$^{2+}$-sensitive barrel (1) of the leading electrode are shown. These Ca$^{2+}$ signals were measured near the outer segment in response to flashes of increasing strength. Like the photosignals, the Ca$^{2+}$ signals show a time course that is dependent on the flash strength. However, the time courses are obviously completely different: If nonsaturating flashes are applied, the extracellular Ca$^{2+}$ concentration increases slowly and reaches a maximum amplitude shortly after the photosignal has passed its maximum. Enhancing the flash strength increases the initial slope rate and the maximum amplitude of the Ca$^{2+}$ signal. If saturating flashes are applied, the Ca$^{2+}$ concentration increases with a maximum initial slope along a common time course, reaching a maximum amplitude when the plateau

The diagram in Fig. 5 c represents the initial slope of the Ca$^{2+}$ signal as a function of the maximum photosignal amplitude. Surprisingly, the Ca$^{2+}$ concentration increases with an initial rate that is correlated by a linear function with the maximum of the photosignal. The reason for this remarkable relation is not known. However, it corroborates the evidence given above: When the dark current is completely suppressed by a saturating flash the external Ca$^{2+}$ concentration, [Ca]$\text{o}$, increases with a maximum initial slope (see Fig. 5 b).

The closure of the membrane channels occurs faster than the external Ca$^{2+}$ concentration increases (at least six times, cf. Fig. 5, a

![Net Ca$^{2+}$ efflux](image1)

![Ca$^{2+}$ extruded](image2)

**FIGURE 6.** (a) Net Ca$^{2+}$ efflux $Q_z$ from the rod (per unit length) determined from the light-induced increment $\Delta[Ca_z]$ of the external Ca$^{2+}$ concentration given at different electrode positions $z$ (see Fig. 4). The retina was stimulated at $t = 0$ by a flash exciting 500 R°/ros. The temperature was 30°C. The Ringer's solution contained 200 $\mu$M Ca$^{2+}$. (b) Amount of Ca$^{2+}$ ions $\Delta Ca_z$ extruded from the rod (per unit length). $\Delta Ca_z$ was obtained by integration of the corresponding Ca$^{2+}$ efflux $Q_z$ (a, left column) over the time $t$. $Q_z$ and $\Delta Ca_z$ are depicted as a function of time $t$ after the flash.
and b), so that, after saturating flashes, the rate of Ca²⁺ increase is not diminished by any Ca²⁺ influx.

If small diffusion losses from the external space of the outer segment area are neglected, the initial rate of Ca²⁺ increase can be directly related to the initial rate of Ca²⁺ release from the outer segment, i.e., the net Ca²⁺ efflux Q_{os}. In conclusion, Fig. 5 c indicates that, in any case, the initial net Ca²⁺ efflux Q_{os} increases with the flash strength, yielding a maximum when the photosignal approaches saturation.

Ca²⁺ Sources at Distinct Positions along the Rod

From the time- and space-dependent changes of the extracellular Ca²⁺ concentration as presented in Fig. 4, the source function q(z,t) was calculated as described under Theory in Materials and Methods (see above).

Finally, Q_{os}(z,t) was derived from the source function that represents the net Ca²⁺ efflux per unit length of the rod at each microelectrode position z, and at any time t after the flash (cf. Eq. 6).

Fig. 6 a shows the time course of the net Ca²⁺ efflux Q_{os}(z,t) in penetration depths between 4 and 44 μm, which in the following considerations is regarded as a measure for the source function. In Ringer’s solution just above the retina (z < 0), the source function is found to remain zero after the flash (not shown), proving that the change of the Ca²⁺ concentration above the retina is solely attributed to diffusion. Along the outer segments, however, immediately after the flash, Ca²⁺ sources appear. After reopening of the Na⁺ channels in the plasma membrane of the outer segments, the sources vanish again and sinks occur temporarily. Finally, the source function becomes zero again, i.e., the dark state is reestablished.

From the inner segment up to the synapse, only Ca²⁺ sinks are found to appear after the flash (in Fig. 6 a, shown up to a depth of 44 μm). These sinks are not sensitive to the Ca²⁺ channel blocker verapamil (not shown). The appearance of Ca²⁺ sinks at the synapse suggests that the dramatic increase of the Ca²⁺ concentration in the synaptical region as shown in Fig. 4 must be due to diffusion. However, as already pointed out, the increase is not due to a diffusion of Ca²⁺ ions from the outer segments to the synaptical region.

The time integral \( \int Q(t) \, dt \) of the Ca²⁺ efflux yields the net amount of Ca²⁺ ions \( \Delta C_{a} \) pumped out of the photoreceptor at a depth \( z \) during the time \( t \) after the onset of light stimulation. Fig. 6 b illustrates \( \Delta C_{a} \) as a function of time \( t \) after the flash determined at distinct penetration depths \( z \). Along the outer segment, \( \Delta C_{a} \) reaches a maximum when the Na⁺ channels start to reopen. The subsequent decline of \( \Delta C_{a} \) indicates that Ca²⁺ ions flow back into the outer segment. Whereas, at the tip, the same amount of Ca²⁺ pumped out during the photosresponse flows back after reopening of

**Figure 7.** (a) Ca²⁺ influx into the outer segment, \( J_{in} \), normalized to the dark value \( J_{in}^{d} \). Since \( J_{in} \) is a constant fraction of the dark current, it is deduced from the photosignal by the equation \( J_{in} = J_{in}^{d} \cdot (A - A_{max}) / A_{max} \), where \( A \) and \( A_{max} \) are amplitude and peak amplitude of the photosignal, respectively. The saturated photosignal was measured at a penetration depth \( z = 28 \mu m \); (b) net Ca²⁺ efflux \( Q_{os} \) from the outer segment normalized to \( J_{in}^{d} \). (c) Net amount \( \Delta C_{os} \) of Ca²⁺ ions extruded from the whole outer segment obtained by integration of \( Q_{os} \) (trace b), and (d) time course of the intracellular Ca²⁺ concentration \( [Ca^{2+}] \), normalized to the dark value \( [Ca^{2+}]^{d} \) derived from traces a and b as described in the Discussion. For calibration, it was presupposed that the initial value \( Q_{0} \) of net efflux \( Q_{os} \) is given by \( Q_{0} = B \cdot J_{in}^{d} \). The activation factor \( B = 1.37 \) allows for the activation of the Na⁺/K⁺-Ca²⁺ exchanger. This activation is assumed to be caused by the hyperpolarization that follows the dark current shut-off after a saturating flash (Requena, 1983) and disappears again during dark current regeneration. As in the dark, one has \( J_{in} = J_{in}^{d} \), it follows \( Q_{0} = 1.37 J_{in}^{d} \), which is used as calibration for Fig. 7 d. All traces a–d are plotted versus the time \( t \) after the flash. The retina was illuminated at \( t = 0 \) by a saturating flash exciting >250 R/μs. The Ringer’s solution contained 250 μM Ca²⁺, the temperature was 23°C.

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the Na⁺ channels, surprising, only a part of it is re-
stored in the middle and at the proximal end of the
outer segment.

After the dark state is reestablished, the outer seg-
ment seems to have lost whereas the other compart-
ments of the rod seem to have taken up a considerable
amount of Ca²⁺ ions.

Net Ca²⁺ Efflux Q₀ₙ from the Outer Segment

Fig. 7 a shows a photosignal derived at z = 28 μM after
an intense flash of light that caused the dark current to
be completely suppressed for ~7.5 s. As shown above,
time course of the circulating receptor current is
sufficiently described by the photovoltage measured extracellulary. As a certain percentage of the inward membrane current is carried by Ca²⁺ ions (see below), the time course of the Ca²⁺ influx into the outer segment is represented directly by the time course of the photosignal. The corresponding net Ca²⁺ efflux Q₀ₙ from the total outer segment after the flash is shown in
Fig. 7 b. Q₀ₙ is the axial integral of Q₀ₙ between z = 0 and z = 25 μm. The net Ca²⁺ efflux shown in Fig. 7 b
reaches a maximum of 1.2 × 10⁵ Ca²⁺/s immediately
after the flash. Subsequently, it declines exponentially
with a time constant τ₀ₙ of 4 s as long as the dark
current is completely suppressed. At the moment the
dark current begins to regenerate, the net efflux is fol-
lowed by a temporary net influx indicating a restora-
tion of Ca²⁺ ions in the depleted outer segment. Simi-
lar results were obtained from the rod of the toad
Bufo marinus (Miller and Korenbrot, 1987) and from the
bullfrog retina (Gold, 1986).

Fig. 7 c shows the amount ΔCa₀ₙ of Ca²⁺ ions totally
released from the whole outer segment up to the time t
after the flash, which is obtained by time integration of
Q₀ₙ shown in Fig. 7 b as ΔCa₀ₙ = ∫₀ₙ Q₀ₙ dt. A maximum
of 2.8 × 10⁵ Ca²⁺ are liberated from the outer segment
during the photosponse. However, only half of this
amount is restored when the dark level is regained.

Fig. 7 d represents the corresponding time depen-
dence of the intracellular Ca²⁺ concentration [Ca]ᵢ,
which was calculated as described in detail in the leg-
end of Fig. 7 and in the Discussion. In principle, the
time course of [Ca]ᵢ derived from the Ca²⁺ efflux
via the Na⁺/K⁺–Ca²⁺ exchanger, which is assumed to
be proportional to [Ca]ᵢ. The Ca²⁺ efflux, however, re-
sults from the difference between net Ca²⁺ efflux (trace
7 b) and Ca²⁺ influx (trace 7 a). Because of this proce-
dure, the time course of [Ca]ᵢ is not directly related to
ΔCa₀ₙ as shown in Fig. 7 c. It is conspicuous that the in-
ternal Ca²⁺ concentration is found to return to the pre-
stimulus dark level, although the extruded Ca²⁺ does
not completely flow back into the outer segment. This
surprising effect will be a subject of the Discussion.

Variation of the Ca²⁺ Concentration in Ringer’s Solution

The net Ca²⁺ efflux Q₀ₙ from the outer segment was
determined for different concentrations in Ringer’s so-
lution by using the same piece of retina. Fig. 8 a shows
the net Ca²⁺ efflux Q₀ₙ as a function of time after the
flash at 0.2, 0.5, and 1 mM Ca²⁺ in Ringer’s solution. A
fourth trace obtained at 0.1 mM Ca²⁺ was omitted in Fig. 8 a.
for clarity. The magnitude of $Q_{os}$ increases with the external Ca$^{2+}$ concentration in Ringer’s solution. However, the time course of $Q_{os}$ is independent thereof.

Corresponding properties are revealed by the time integral $\Delta C_{aos}$ of the net Ca$^{2+}$ efflux, which is plotted in Fig. 8 a as a function of time after the flash. It is obvious that the magnitude of $\Delta C_{aos}$ is dependent on the external Ca$^{2+}$ concentration, whereas the time course is not. It is noteworthy that this experiment shows again only half of the released Ca$^{2+}$ to be taken back up by the outer segment (cf. also Fig. 7 c).

In Fig. 8 c, the maximum of the net Ca$^{2+}$ efflux $Q_{os}^{max}$ as well as the maximum of Ca$^{2+}$ extruded from the outer segment, $\Delta C_{aos}^{max}$, are plotted in dependence of the extracellular Ca$^{2+}$ concentration. In this diagram, the corresponding $Q_{o}$ values of the initial net Ca$^{2+}$ efflux, which are derived as described in the next section, are also plotted. All three quantities show a nearly linear increase with the Ca$^{2+}$ concentration in Ringer’s solution.

**Variation of Flash Intensity and Temperature**

Fig. 9 represents the net Ca$^{2+}$ efflux $Q_{os}$ from the outer segment and the respective photosignals determined at 23 and 30°C. Saturating flashes exciting 600 and 3,000 R*/ros were used. The maximum Ca$^{2+}$ efflux $Q_{os}^{max}$ appears with a distinct delay of 0.5–1.0 s after the saturating flash. On the other hand, Miller and Korenbrot (1987) showed a net Ca$^{2+}$ efflux from amphibian rod outer segments which rises instantly, i.e., within at least 0.1 s after a saturating flash to a maximum value. There is no weighty reason as yet to assume that the net Ca$^{2+}$ efflux in mammalian rods rises much slower. Therefore, the delayed appearance of $Q_{os}^{max}$ as shown in Fig. 9 should be solely due to the band width limitation caused by the Ca$^{2+}$-sensitive electrode and the smoothing procedure.

Therefore, the actual net Ca$^{2+}$ efflux $Q_{os}$ was approximated by a maximum initial value $Q_{o}$, which is reached instantly after the flash at $t = 0$ and decays exponentially to zero with a time constant $\tau_{Q}$ as long as the Ca$^{2+}$ influx is completely suppressed:

$$Q_{os} = Q_{o} e^{-t/\tau_{Q}}$$

(11)

Instead of Eq. 11, according to the bandwidth limitation characterized by an effective time constant $\tau_{b}$, the measured $Q_{os}$ signal is more accurately described by

$$Q_{os} = Q_{o} \frac{\tau_{Q}}{\tau_{Q} - \tau_{b}} (e^{-t/\tau_{Q}} - e^{-t/\tau_{b}}).$$

(12)

In principle, $Q_{o}$ and $\tau_{Q}$ can be derived from the given $Q_{os}$ data by fitting Eq. 12 to the first part of the signals shown in Fig. 9 or 7 b. However, according to the low S/N ratio of these signals, the data obtained by this curve fitting procedure are subject to considerable error. Thus, for data evaluation, the following approximation procedure was used: As shown in Fig. 9 by dashed lines, monoexponential curves were fitted to the measured $Q_{os}$ signals in the time period between $t_{max}$ and three to four times $t_{max}$. The initial value $Q_{o}$ of the Ca$^{2+}$ efflux was then determined by extrapolating the exponential fit curve to $t = 0$, i.e., to the moment the flash was applied. By model calculation, it was...
shown that, by this simple approximation, even in the most unfavorable case \( \tau_B = \tau_Q \), the extrapolated value of \( Q_{os} \) is only slightly underestimated (down to maximally \(-30\%\)), whereas the time constant \( \tau_Q \) is overestimated (up to maximally \(35\%\)).

The results of the signal data evaluation are summarized in Table II: The maximum of the net \( \text{Ca}^{2+} \) efflux, \( Q_{os}^{\text{max}} \), as well as the time constant \( \tau_Q \) of its exponential decay, are dependent on the temperature and independent of the flash intensity \( I_F \). From the \( Q_{os}(t) \) traces represented in Fig. 9, the maximum rate \( Q_{os}^{\text{max}} \) of the \( \text{Ca}^{2+} \) efflux from the outer segment was determined to be in the average \(-6 \cdot 10^4\) and \(9 \cdot 10^4\) \(\text{Ca}^{2+}/\text{s}\), and the time constant \( \tau_Q \) was determined to be \(3.4\) and \(0.8\) s at a temperature of \(23\) and \(30\){\textdegree}C, respectively. The average value of the time constant was \(4.2\) \((\pm1.2\) SD, \(n = 5\)) s at \(23\){\textdegree}C and \(1.0\) \((\pm0.2\) SD, \(n = 4\)) s at \(30\){\textdegree}C, showing a temperature dependence corresponding to an activation energy of \(150 \pm 45\) kJ/mol. Thus, at the physiological temperature of \(37\){\textdegree}C, the decay time is calculated to be \(\tau_Q = 0.3\) s.

Initial values \(Q_o\) are given in Table II for temperatures of \(23\) and \(30\){\textdegree}C and in Table III also for different values of the external \(\text{Ca}^{2+}\) concentration. As expected, it is evident from Table II that, like \(Q_{os}^{\text{max}}\), the initial value \(Q_o\) is also independent of the flash intensity but rises with temperature and, according to Fig. 8 e, also with the external \(\text{Ca}^{2+}\) concentration.

The maximum amount of \(\text{Ca}^{2+}\) ions, \(\Delta Q_{os}\), that the \(\text{Na}^+/\text{K}^+ - \text{Ca}^{2+}\) exchanger is able to pump out of the outer segment if the \(\text{Ca}^{2+}\) influx is indefinitely suppressed, i.e., if no \(\text{Ca}^{2+}\) flows back, is obtained by the infinite time integration of Eq. 11, yielding

\[
\Delta Q_{os}^\infty = \tau_Q \cdot Q_o.
\]

Determined values of \(\Delta Q_{os}^\infty\) are given in Table II. It is noteworthy that \(\Delta Q_{os}^\infty\) is reduced by \(\sim 55\%\) if the temperature is increased from \(23\) to \(30\){\textdegree}C.

### Determination of Net \(\text{Ca}^{2+}\) Efflux from the Outer Segment at Steady Light

After the onset of a constant illumination, only a transient increase of the extracellular \(\text{Ca}^{2+}\) concentration was expected. After some time of saturating illumination, the outer segments were expected to lose their \(\text{Ca}^{2+}\) contents completely so that the \(\text{Ca}^{2+}\) efflux should vanish. Thereupon, the elevated concentration between the photoreceptors should disappear because by diffusion, the \(\text{Ca}^{2+}\) ions should be equally distributed within the Ringer’s solution.

Contrary to expectations, a constant rise of the extracellular \(\text{Ca}^{2+}\) concentration is produced by illuminating the retina with steady light. Such a continuous elevation could reliably be detected at least up to \(5\) min. An experiment performed with continuous illumination is shown in Fig. 10: The retina was illuminated for \(10.5\) s with steady light that completely shuts off the dark current (a). The external \(\text{Ca}^{2+}\) concentration (b) is permanently elevated by \(\sim 2\) \(\mu\)M, indicating a continuous net \(\text{Ca}^{2+}\) efflux from the outer segments during steady illumination. The net \(\text{Ca}^{2+}\) efflux (c) corroborates this indication: The \(\text{Ca}^{2+}\) efflux from the outer segment rises to a maximum value after the onset of the illumination and subsequently declines exponentially to a resting level, which by exponential curve fitting was found to be \(\sim 13\%\) of the maximum value. The values of the maximum \(\text{Ca}^{2+}\) efflux \((1.5 \cdot 10^5 \text{Ca}^{2+}/\text{s})\) and the time constant of the exponential decline \((\tau_Q = 2.5\) s) are of the same order of magnitude as those obtained using saturating flashes. The corresponding amount of extruded \(\text{Ca}^{2+}\), \(\Delta Q_{os}\), is shown in Fig. 10 d.

It is striking that, after switching off the light, only \(30\%\) of the \(\text{Ca}^{2+}\) extruded before from the outer segment is restored. That means the \(\text{Ca}^{2+}\) deficiency of the outer segment is higher after long-lasting steady illumination than after intense saturating flashes (cf. \(50\%\) in Fig. 8 b).

### Discussion

The light-induced reduction of the photovoltage that is proportional to the dark current and the increase of the \(\text{Ca}^{2+}\) concentration were measured extracellularly in the photoreceptor layer of the albinot rat retina. For stimulation, flashes and steady light were used. By applying the one-dimensional diffusion equation (Eq. 4) to the measured data, the \(\text{Ca}^{2+}\) source function and the corresponding net \(\text{Ca}^{2+}\) efflux along the rod were directly derived. Unlike the study of Yoshikami et al. (1980), it was not necessary to make simplifying assumptions concerning the distribution of \(\text{Ca}^{2+}\) sources along the rod and about the extracellular space in the photoreceptor layer.
Fluxes and Channel Regulation in Rat Rods

The method used in this study enables the determination of Ca²⁺ fluxes through the plasma membrane at distinct positions of the rods during a complete phototransduction. By this method, at an extracellular Ca²⁺ concentration of 250 μM, fluxes down to 1 · 10⁴ Ca²⁺/s per μm rod length can be resolved. The latter is equivalent to an exchange current of 3 fA per rod if one allows for a transport of one electron charge per Ca²⁺ transport. Therefore, even small Ca²⁺ fluxes could be measured which cannot be resolved by suction electrodes having a resolution of 200 fA (Miller and Korenbrot, 1987; Nakatani and Yau, 1988; Tamura et al., 1991).

Our investigations of rat rods reveal Ca²⁺ fluxes that are large in extent but behave similarly to those in amphibian rods. However, there are some discrepancies and certain important new findings.

**Light-induced Reduction of the Ca²⁺ Concentration in the Outer Segment**

In the dark, there is no net Ca²⁺ efflux from the outer segment. This is indicated for instance by the prestimulus zero value of Q₉₀ shown in Fig. 10 c. Moreover, if the Ca²⁺ sensitive electrode is inserted in the dark into the photoreceptor layer, there is no Ca²⁺ gradient to be detected between the outer segment region and the retinal layer above the retina (Rüppel and Cieslik, 1988). This is presumed to be due to a balance between a Ca²⁺ influx through the cGMP-dependent membrane channels and a Ca²⁺ efflux via the Na⁺/K⁺–Ca²⁺ exchanger (Yau and Nakatani, 1985; Miller and Korenbrot, 1987). When exposed to light, however, a net Ca²⁺ efflux is evoked from the outer segment, i.e., the Ca²⁺ efflux exceeds the Ca²⁺ influx. This is indicated by the increase of the extracellular Ca²⁺ concentration and is directly shown by the source function rising from zero to positive values. Most probably, the light-induced net Ca²⁺ efflux is almost exclusively due to a reduced Ca²⁺ influx.

To study the Ca²⁺ fluxes in more detail, flashes of increasing light intensity were applied to the rat retina. In Fig. 5 it is shown that the net Ca²⁺ efflux approaches a maximum value if the maximum amplitude of the photosignal reaches the saturating level. In this case, the Ca²⁺ influx through the membrane channels into the outer segment is completely interrupted. This result agrees with findings of Gold (1986) and Miller and Korenbrot (1987), who also obtained a maximum Ca²⁺ efflux from amphibian rods when all of the Na⁺ channels close after a saturating flash.

The maximum of the net Ca²⁺ efflux appears immediately after a saturating flash. Subsequently, as long as the dark current is shut off, the Ca²⁺ efflux decays exponentially (see Figs. 7 and 9). The maximum value and the time constant of the exponential decline are independent of the saturating flash strength. During this period of time, the Ca²⁺ influx through the outer segment is completely interrupted. Thus, the exponential decline of the net Ca²⁺ efflux might be due to a corresponding exponential reduction of the free Ca²⁺ concentration in the outer segment (see also Miller and Korenbrot, 1987), since the magnitude of the Ca²⁺ extrusion is found to be proportional to the free cytoplasmic concentration (Lagnado et al., 1988).

A reduced Ca²⁺ concentration in the outer segments becomes apparent, indeed, when the cGMP-dependent membrane channels reopen. Then the outer segment acts as a sink so that the Ca²⁺ concentration near the outer segment falls even below the dark level. Obviously, the Ca²⁺ influx exceeds the Ca²⁺ efflux at this stage. This is the result of a decreased Ca²⁺ efflux caused by a reduced Ca²⁺ concentration in the outer segment. Other explanations of the transient appearance of sinks may be a light-induced increase of Ca²⁺ permeability of the membrane channels or an inhibition of the Na⁺/K⁺–Ca²⁺ exchange mechanism. However, at present there is no indication for either. As shown below, the Ca²⁺ permeability stays constant.
By means of the Ca\textsuperscript{2+} influx exceeding the Ca\textsuperscript{2+} efflux, the reduced intracellular Ca\textsuperscript{2+} concentration is restored. When the free Ca\textsuperscript{2+} concentration in the outer segment reaches the dark level, the Ca\textsuperscript{2+} influx and efflux are balanced again.

In summary, the results presented here agree with the notion that light decreases the Ca\textsuperscript{2+} influx through the cGMP-dependent ion channels, whereas the photosresponse, the unidirectional Ca\textsuperscript{2+} efflux via the Na\textsuperscript{+}/K\textsuperscript{+}-Ca\textsuperscript{2+} exchanger reduces the Ca\textsuperscript{2+} concentration in the outer segment. The results obtained here from rat rods are in accordance with the present concept for the light-induced changes of Ca\textsuperscript{2+} fluxes across the plasma membrane of amphibian rods. Therefore, it seems to be feasible to apply to these data of rat rods a model that was discussed for ion fluxes in amphibians (Miller and Korenbrot, 1987). In this model the Ca\textsuperscript{2+} influx \( (J_n) \) is assumed to parallel the dark current, whereas the Ca\textsuperscript{2+} efflux \( (J_{eff}) \) via the Na\textsuperscript{+}/K\textsuperscript{+}-Ca\textsuperscript{2+} exchanger is proportional to the free Ca\textsuperscript{2+} concentration \([Ca]\) in the outer segment and is affected by hyperpolarization. As the net Ca\textsuperscript{2+} efflux \( Q_{QS} \) is the sum of Ca\textsuperscript{2+} in- and efflux, \( Q_{OS} = J_n + J_{eff} \), the Ca\textsuperscript{2+} efflux \( J_{eff} \) can be readily deduced from \( Q_{OS} \) (Fig. 7 b) by subtracting the Ca\textsuperscript{2+} influx \( J_n \), which corresponds to the photosignal (Fig. 7 a, see legend of Fig. 7 b for the scaling factor). The Ca\textsuperscript{2+} efflux \( J_{eff} \) provides the free Ca\textsuperscript{2+} concentration \([Ca]_f\) after a saturating flash (Fig. 7 d), which is exponentially reduced to a minimum of ~35% of the dark value \([Ca]^d\). This result fits well with fluorochromic measurements of the free intracellular Ca\textsuperscript{2+} concentration with Fura II of Ratto et al. (1988) but partially disagrees with recent results of McCarthy et al. (1994).

**Dependence of Maximum Net Ca\textsuperscript{2+} Efflux on the External Ca\textsuperscript{2+} Concentration**

As shown in Table III, the initial maximum Ca\textsuperscript{2+} efflux \( Q \) from the outer segment decreases by a factor of 3.6 if the Ca\textsuperscript{2+} concentration in the Ringer’s solution is reduced from 1 to 0.1 mM. This should correspond to a decrease of the Ca\textsuperscript{2+} influx in the dark by the same amount. As the Ca\textsuperscript{2+} efflux is proportional to the free Ca\textsuperscript{2+} concentration \([Ca]_f\) in the outer segment (Lagnado et al., 1988), \([Ca]_f\) should be small compared with the \( K_m \) value for the Ca\textsuperscript{2+} extrusion by the Na\textsuperscript{+}/K\textsuperscript{+}-Ca\textsuperscript{2+} exchanger. Thus, the observed decrease of the maximum Ca\textsuperscript{2+} efflux should indicate that the dark value of the free Ca\textsuperscript{2+} concentration \([Ca]_f\) has dropped by a factor of 3.6 as well. This is valid only if the internal concentrations of Na\textsuperscript{+} and K\textsuperscript{+} as well as the membrane voltage remain unaffected by the alteration of the external Ca\textsuperscript{2+} concentration. Actually, however, decreasing the external Ca\textsuperscript{2+} concentration increases the dark current. As a consequence, the Na\textsuperscript{+} concentration in the outer segment should be elevated. This in turn would result in a deactivation of the Na\textsuperscript{+}/K\textsuperscript{+}-Ca\textsuperscript{2+} exchange mechanism (Hodgkin and Nunn, 1987) so that the decreased Ca\textsuperscript{2+} efflux could be attributed also to a rise of the Na\textsuperscript{+} concentration in the outer segment. On the other hand, an evaluation of the \( Q_{OS}(t) \) traces as shown in Fig. 8 a yielded the rate constant \( 1/\tau_Q \) of the Ca\textsuperscript{2+} extrusion to be independent of the external Ca\textsuperscript{2+} concentration. Because \( 1/\tau_Q \) depends on the internal Na\textsuperscript{+} concentration (Hodgkin and Nunn, 1987), this finding suggests that the internal Na\textsuperscript{+} concentration remains unaffected by an increase of the external Ca\textsuperscript{2+}.
concentration \([\text{Ca}]_o\). Thus, as a conclusion, the decrease of the \(\text{Ca}^{2+}\) efflux can be regarded to be mainly due to a fall of the free intracellular \(\text{Ca}^{2+}\) concentration \([\text{Ca}]_f\).

Recent measurements in amphibian rod outer segments revealed \([\text{Ca}]_f\) to be \(~300-400\) nM at an external \(\text{Ca}^{2+}\) concentration of \(1\) mM (Miller and Korenbrot, 1989; Lagnado et al., 1992). It must be emphasized that this result is quite difficult to obtain. For the much smaller mammalian rods, the corresponding data are not yet available. Now, as several properties of amphibian and mammalian rods are shown to agree (Nakatani et al., 1991; Tamura et al. 1991), it may be justified to assume that \([\text{Ca}]_f\) in rat rods is not much different from that in amphibian rods. Therefore, if reduced by a factor of 3.6 when lowering the extracellular \(\text{Ca}^{2+}\) concentration from 1.0 to 0.1 mM, \([\text{Ca}]_f\) should drop in the rat rod outer segment from 300-400 nM to 80-110 nM. The lower value seems to be relatively unlikely because, according to Koch and Stryer (1988), the GC should be close to full activation at \([\text{Ca}]_f = 80\) nM. However, such a GC activation should increase the cGMP level dramatically, followed by an opening of additional membrane channels and a considerable inflow of \(\text{Na}^+\). The resulting increase of the dark current should far overcome the observed value (cf. Table III). Furthermore, no efficient desensitization would be expected in the rod receptor at \([\text{Ca}]_o <0.1\) mM. Actually, an effective adaptation is still observed at this external \(\text{Ca}^{2+}\) concentration. In summary, it seems to be more realistic to assume the upper limit of \(400\) nM to be the dark value of \([\text{Ca}]_f\) at \([\text{Ca}]_o = 1\) mM. In this case, \([\text{Ca}]_f\) is reduced only to 110 nM so that nothing but a moderate GC activation is expected if the external \(\text{Ca}^{2+}\) concentration is reduced from 1 to 0.1 mM.

**Dependence of the Net \(\text{Ca}^{2+}\) Efflux on the Temperature**

According to Table II, the maximum of the net \(\text{Ca}^{2+}\) efflux as well as its exponential decline in time are strongly temperature dependent. If the temperature is raised from 23 to 30°C, the maximal \(\text{Ca}^{2+}\) efflux increases on the average by 70%, whereas the decay time constant is reduced by \(~25\%\). It is well known that a rise in temperature increases the dark current (Penn and Hagins, 1972; Robinson et al., 1993). If the \(\text{Ca}^{2+}\) selectivity of the ion channels remains unaffected, an increased dark current implies an enhanced \(\text{Ca}^{2+}\) influx into the outer segment. However, this need not lead to an increased dark level of the free \(\text{Ca}^{2+}\) concentration \([\text{Ca}]_f\) if the increase in the dark current is accompanied by a temperature-dependent activation of the \(\text{Na}^+/\text{K}^+-\text{Ca}^{2+}\) exchange mechanism. Moreover, if the activation of the \(\text{Ca}^{2+}\) efflux exceeds the enhancement of the \(\text{Ca}^{2+}\) influx, the dark level \([\text{Ca}]_f\) would even fall. In any case, an increased peak maximum of the net \(\text{Ca}^{2+}\) efflux would be expected.

As the exponential decline of the net \(\text{Ca}^{2+}\) efflux occurs only during completely interrupted dark current its time constant is solely dependent on the activity of the \(\text{Na}^+/\text{K}^+-\text{Ca}^{2+}\) exchanger. Thus, the large temperature change of the decay rate \(1/\tau_D\), which corresponds to an activation energy of 150 kJ/mol, must be due to the temperature dependence of the exchanger activity. The much smaller temperature dependence of the maximal \(\text{Ca}^{2+}\) efflux \(Q_o\), however, is due to the fact that \(Q_o\) represents the dark equilibrium between \(\text{Ca}^{2+}\) in- and efflux and thus reflects the combined temperature dependencies of both processes.

**Deficiency in the Balance of \(\text{Ca}^{2+}\) Fluxes**

By numerous experiments, it has been repeatedly shown (cf. Figs. 6–8) that only a fraction of the \(\text{Ca}^{2+}\) released during a photosresponse will be taken back up by the rod outer segment when the dark-adapted state is reached again. This unexpected effect is observed if light stimulation is achieved either by a saturating flash (Fig. 9) or by a saturating long pulse of steady light (Fig. 10). Apparently, saturating light stimulation induces an irreversible loss of \(\text{Ca}^{2+}\) ions from the outer segment.

Such an irreversible light-induced loss of \(\text{Ca}^{2+}\) from the outer segment of the rat rod has only been reported before by Yoshikami et al. (1980), who used similar experimental conditions. However, such an effect was not obtained from rods or retinas of amphibians (Miller and Korenbrot, 1987; Gold, 1986). This discrepancy may be due to the different methods used or to differences between species.

Actually, the \(\text{Ca}^{2+}\) deficiency in the rod outer segment detected by applying strong flashes amounts to \(~50\%\) (cf. Figs. 7 and 8). A higher deficiency, however, was evoked by long-lasting and saturating steady light (e.g., 70% in Fig. 10 d). The largest deficiency measured in this study was as high as 95%. Therefore, the magnitude of the deficiency is obviously dependent on the duration of the plateau phase of the saturating photosresponse and is thus related to the amount of \(\text{Ca}^{2+}\) extruded during this time period (cf. Fig. 10).

During long periods of continuously interrupted dark current, another phenomenon that is probably correlated with the irreversible \(\text{Ca}^{2+}\) release becomes evident: After very intense flashes (see Fig. 5 b) and during saturating steady light (see Fig. 10 b), a constantly elevated \(\text{Ca}^{2+}\) concentration is observed in the extracellular space between the rod outer segments, indicating a continuous \(\text{Ca}^{2+}\) release from the outer segment. Correspondingly, the net \(\text{Ca}^{2+}\) efflux during steady light does not disappear but resumes a residual value (see Fig. 10 c, extrapolation).
The origin of these two conspicuous findings is yet to be established. On the basis of the various results obtained in this study, we suggest, that during a photoresponse, Ca$^{2+}$ is liberated from large internal stores within the rod into the cytoplasm of the outer segment. From there, the Ca$^{2+}$ ions are extruded into the extracellular space. A part of these stores is assumed to release Ca$^{2+}$ irreversibly by this process. The residual Ca$^{2+}$ efflux during steady light as shown in Fig. 10 c is suggested to reflect the irreversible Ca$^{2+}$ extrusion.

An estimation may confirm this suggestion and give indications to distinguish between reversible and irreversible Ca$^{2+}$ extrusion during long term steady illumination. In Fig. 10 d, $\sim$70% or 3.5 $\cdot$ 10$^5$ of the Ca$^{2+}$ ions extruded from the outer segment appear to be irreversibly released. Supposing that Ca$^{2+}$ is irreversibly extruded at a constant rate during the whole time period of the dark current reduction ($\sim$13 s), the mean rate of irreversible Ca$^{2+}$ release amounts to 3.5 $\cdot$ 10$^5$/13 s = 2.7 $\cdot$ 10$^4$ Ca$^{2+}$/s. In fact, this value corresponds quite well with the residual Ca$^{2+}$ extrusion rate of (2 $\pm$ 1)$\cdot$10$^4$ Ca$^{2+}$/s, which is derived from Fig. 10 c. Thus, it is concluded that the residual Ca$^{2+}$ efflux might be due to the irreversible Ca$^{2+}$ release, whereas the exponentially decaying peak initially superposed to the residual efflux should represent the reversible Ca$^{2+}$ extrusion.

In more detail, the following mechanisms may account for an irreversible Ca$^{2+}$ liberation into the cytoplasm during a photoresponse: (a) Endogenous Ca$^{2+}$ buffers within the outer segment release Ca$^{2+}$ either irreversibly or, after the release, the buffers rebind Ca$^{2+}$ very slowly. (b) The disks in the outer segment act as Ca$^{2+}$ stores and release Ca$^{2+}$ irreversibly or they take up Ca$^{2+}$ very slowly. Actually, these disks are known to contain large amounts of Ca$^{2+}$ (Hagins and Yoshikami, 1975; Somlyo and Walz, 1985; Fain and Schröder, 1985). (c) The inner segment acts as a Ca$^{2+}$ store: Ca$^{2+}$ ions diffuse from the inner to the outer segment driven by a Ca$^{2+}$ gradient caused by the light-induced reduction of the free Ca$^{2+}$ concentration in the outer segment. The Ca$^{2+}$ gradient is maintained by a slightly higher Ca$^{2+}$ concentration in the inner segment, kept constant by a Ca$^{2+}$ release from the mitochondria or the endoplasmatic reticulum. Both cell bodies are known to contain large amounts of Ca$^{2+}$ ions (Somlyo and Walz, 1985; Ungar et al., 1984). (d) An influx of Ca$^{2+}$ ions from the extracellular space into the inner segment is followed by a diffusion into the outer segment, from which they are extruded again into the interstitial space. Thus, during a photoresponse, a circulating flow of Ca$^{2+}$ ions results between the outer and inner segment that is opposite to the circulating Na$^+$ current in the dark.

At present, it is not possible to favor one of these mechanisms (a–d) because little is known about the distribution and the light-induced fluxes of Ca$^{2+}$ ions within the rod. Nevertheless, several results support a diffusion of Ca$^{2+}$ ions from the inner to the outer segment during a photoresponse. The major deficiency of Ca$^{2+}$ was measured in the middle and at the proximal end of the outer segment, whereas there is almost no deficiency at the tip of the outer segment, most distant from the inner segment (see Fig. 6 b). This means that most of the irreversibly released, i.e., not reentered, Ca$^{2+}$ ions were extruded from the part of the outer segment that is next to the inner segment. This should be expected if the inner segment is the source of the irreversible Ca$^{2+}$ liberation as suggested by mechanism c.

Furthermore, as shown in Fig. 6 a during the first 3–4 s after the flash, Ca$^{2+}$ sinks are revealed along the inner segment whereas Ca$^{2+}$ sources are observed along the outer segment. This is in accordance with mechanism d.

On the other hand, the Ca$^{2+}$ extrusion experiments with steady light (Fig. 10) do not disprove unequivocally the possibility that the Ca$^{2+}$ buffers in the outer segment might be the source for the observed Ca$^{2+}$ deficiency as postulated in models a and b. This is easily shown by the following estimation: Adopting a buffer capacity of 2 Ca$^{2+}$/rhodopsin (Hagins and Yoshikami, 1975; Schröder and Fain, 1985; Rüppel and Hedrich, unpublished results, 1988), one obtains for the rat rod $\sim$10$^5$ Ca$^{2+}$/rod. Most probably, the disks contain the great majority of Ca$^{2+}$ in the outer segment. This Ca$^{2+}$ reservoir is by far large enough to account for the observed Ca$^{2+}$ deficiency but also for the residual Ca$^{2+}$ efflux detected over a time period of 5 min at most. At the residual efflux rate of 2 $\cdot$ 10$^4$ Ca$^{2+}$/s as given in Fig. 10 c, this reservoir cannot be depleted within a time span of $\sim$1 h.

Furthermore, assuming that during continuous illumination the residual Ca$^{2+}$ efflux of (2 $\pm$ 1)$\cdot$10$^4$ Ca$^{2+}$/s is caused by a Ca$^{2+}$ release from the disks, for 750 disk per rat rod, one obtains an extrusion rate per disk of 15–45 Ca$^{2+}$/s. This value is of the same order of magnitude as the rate of Ca$^{2+}$ exchange in the disks of $\sim$100 Ca$^{2+}$/s per disk, which was found by Fain and Schröder (1987) for the toad retina of Bufo marinus in the dark.

Finally, a constant increase of the extracellular Ca$^{2+}$ concentration during steady light as shown in Fig. 10 b has also been observed by Livsey et al. (1990) in the isolated frog retina. However, if the pigment epithelium was not removed from the photoreceptor layer, an initial increase of the extracellular Ca$^{2+}$ concentration after the onset of steady light was followed by a slow reduction even below the dark level. Therefore, a light-induced Ca$^{2+}$ influx into the pigment epithelium was assumed (Livsey et al., 1990). Obviously, in vivo, during constant illumination the permanent Ca$^{2+}$ efflux from the outer segment feeds a Ca$^{2+}$ current from the outer segment into the pigment epithelium. Because it is a
well-known fact that all trans retinal has to be transferred to the pigment epithelium for regeneration (Rando, 1990; Saari, 1990), it may be speculated that the retinal transport is governed by this light-induced Ca\textsuperscript{2+} current.

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REFERENCES

Allen, M.P., and D.J. Tildesley. 1987. Computer simulation of liquids. Oxford University Press, New York.

Cervetto, L., L. Lagnado, R.J. Perry, D.W. Robinson, and P.A. McNaughton. 1989. Extrusion of calcium from rod outer segments is driven by both sodium and potassium gradients. Nature (Lond.). 317:740-743.

Fain, G.L., and W.H. Schroeder. 1985. Calcium content and calcium exchange in dark adapted toad rods. J. Physiol. (Camb.). 368: 641-655.

Fain, G.L., and W.H. Schroeder. 1987. Calcium in dark-adapted toad rods: evidence for pooling and guanosine-3'5'-monophosphate-dependent release. J. Physiol. (Camb.). 389:361-384.

Friedel, U., G. Wolbrink, P. Wohlfahrt, and N.J. Cook. 1991. The Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger of bovine rod photoreceptors: K\textsuperscript{+}-dependence of the purified and reconstituted protein. Biochim. Biophys. Acta. 1061:247-252.

Gold, G.H. 1986. Plasma membrane calcium fluxes in intact rods are inconsistent with the "calcium hypothesis." Proc. Natl. Acad. Sci. USA. 83:1150-1154.

Hagins, W.A. 1970. The visual process: excitatory mechanisms in the primary receptor cells. In Annual Review of Biophysics and Bioengineering. 1:131-158.

Hagins, W.A., R.D. Penn, and S. Yoshikami. 1970. Dark current and photo current in retinal rods. Biophys. J. 10:380-412.

Hagins, W.A., and S. Yoshikami. 1975. Ionic mechanisms in excitation of photoreceptors. Ann. NY Acad. Sci. 264:314-325.

Hodgkin, A.L., P.A. McNaughton, and B.J. Nunn. 1985. The ionic selectivity and calcium dependence of the light sensitive pathway in rods. J. Physiol. (Camb.). 358:447-468.

Hodgkin, A.L., P.A. McNaughton, and B.J. Nunn. 1987. Measurement of sodium calcium exchange in salamander rods. J. Physiol. (Camb.). 391:347-370.

Hodgkin, A.L., and B.J. Nunn. 1987. The effect of ions on sodium calcium exchange in salamander rods. J. Physiol. (Camb.). 391:371-398.

Hsu, Y.-T., and R.S. Molday. 1993. Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. Nature (Lond.). 361:76-79.

Kawamura, S. 1993. Rhodopsin phosphorylation as a mechanism of cyclic GMP phosphodiesterase regulation by s-modulin. Nature (Lond.). 362:855-857.

Knopp, A. 1994. Kalziumflüsse, Kanalregulierung und Rhodopsindesaktivierung im Vertebraten-Sehstätchen. Berichte aus der Biologie. Verlag Shaker, Aachen, Germany. 110 pp.

Knopp, A., and H. Rüppel. 1995. Is the falling phase of the vertebrate photoreceptor response governed by a Ca\textsuperscript{2+} dependent decay of the activated state of rhodopsin? In Proceedings of the 21st Göttingen Neurobiology Conference. N. Ehner and M. Heisenberg, editors. Georg Thieme, Stuttgart and New York. p. 416.

Koch, K.-W., and L. Stryer. 1988. High cooperative feedback control of retinal rod GC by calcium ions. Nature (Lond.). 334:64-66.

Kuhl, R., H. Rüppel, A. Knopp, R. Hagemann, F.-D. Selke, and H.-P. Berlien. 1995. A comprehensive electrophysiologically study of vertebrate retinae irradiated by low power IR-laser light did not reveal biostimulative effects. Biomed. Lett. 52:7-36.

Lagnado, L., and D.A. Baylor. 1994. Calcium controls light-triggered formation of catalytically active rhodopsin. Nature (Lond.). 367:273-277.

Lagnado, L., L. Cervetto, and P.A. McNaughton. 1988. Ion transport by the Na–Ca exchange in isolated rod outer segments. Proc. Natl. Acad. Sci. USA. 85:4548-4552.

Lagnado, L., L. Cervetto, and P.A. McNaughton. 1992. Calcium homeostasis in the outer segment of retinal rods of the tiger salamander. J. Physiol. (Camb.). 445:111-142.

Lamb, T.D., P.A. McNaughton, and K.-W. Yau. 1981. Spatial spread of activation and background desensitization in toad outer segments. J. Physiol. (Camb.). 319:463-496.

Lisvey, C.T., B. Huang, J. Xu, and C.J. Katrowski. 1990. Light evoked changes in extracellular Ca\textsuperscript{2+}-concentration in frog retina. Vision Res. 30:853-861.

Matthews, H.R. 1991. Incorporation of chelator into guinea-pig rods shows that calcium mediates mammalian light adaptation. J. Physiol. (Camb.). 436:93-105.

Matthews, H.R., R.S. Murphy, G.L. Fain, and T.D. Lamb. 1988. Photoreceptor light adaptation is mediated by cytoplasmic calcium. Nature (Lond.). 334:67-69.

McCarthy, S.T., J.P. Younger, and W.G. Owen. 1994. Free calcium concentrations in bullfrog rods determined in the presence of multiple forms of Fura II. Biophys. J. 67:2076-2089.

Miller, D.L., and J.I. Korenbrot. 1987. Kinetics of light-dependent Ca\textsuperscript{2+} fluxes across the plasma membrane of rod outer segments. J. Gen. Physiol. 90:397-425.

Miller, D.L., and J.I. Korenbrot. 1989. Cytoplasmic free calcium concentration in retinal rod outer segments. Vision Res. 29:939-948.

Nakatani, K., T. Tamura, and K.-W. Yau. 1991. Light adaptation in retinal rods of the rabbit and two other non-primate mammals. J. Gen. Physiol. 97:413-436.

Nakatani, K., and K.-W. Yau. 1988. Calcium and magnesium fluxes across the plasma membrane of the toad rod outer segment. J. Physiol. (Camb.). 395:695-729.

Penn, R.D., and W.A. Hagins. 1969. Signal transmission along retinal rods and the origin of the electroretinographic a-wave. Nature (Lond.). 223:291-295.
retinal rods. *Biophys. J.* 12:1073–1094.

Pepperberg, D.R., J. Jin, and G.J. Jones. 1994. Modulation of transduction gain in light adaptation of retinal rods. *Visual Neurosci.* 11:53–62.

Pugh, E.N., Jr., and J. Altman. 1988. A role for calcium in adaptation. *Nature (Lond.)* 334:16–17.

Pugh, E.N., Jr., and T.D. Lamb. 1990. Cyclic GMP and calcium: the internal messengers of excitation and adaptation in vertebrate rods. *Vision Res.* 30:1923–1948.

Rando, R.R. 1990. The chemistry of vitamin A and vision. *Angew. Chem. Int. Ed. Engl.* 29:461–480.

Ratto, G., R. Payne, W.G. Owen, and R.Y. Tsien. 1988. The concentration of cytosolic free calcium in vertebrate rods measured with FURA-2. *J. Neurosci.* 8:3240–3246.

Requena, J. 1983. Calcium transport and regulation in nerve fibers. *Annu. Rev. Biophys. Bioeng.* 12:257–257.

Robinson, D.W., G. Ratto, L. Lagnado, and P.A. McNaughton. 1993. Temperature dependence of the light response in rat rods. *J. Physiol. (Camb.)* 462:405–481.

Rüppel, H., 1983. Methods for measuring fast reactions. In *Biophysics.* W. Hoppe, W. Lohmann, H. Markl, and H. Ziegler, editors. Springer-Verlag Berlin, Heidelberg, New York, Tokyo. 175.

Rüppel, H., and J. Geslik. 1988. Light stimulated ion fluxes across the plasma membrane of photoreceptors. *Ber. Bunsenges. Phys. Chem.* 92:1020–1025.

Rüppel, H., P. Hochstrat, and H.-E. Buchwald. 1978. Eine gepulste Lumineszenzdiode zur Anregung photochemischer Reaktionen in biologischen Objekten. *Feinwerhtechnik und Meßtechnik.* 86:270–272.

Saari, J.C. 1990. Enzymes and proteins of the mammalian visual cycle. In *Progress in Retinal Research.* N. Osborne and J. Chader, editors. Pergamon Press, Oxford. 363–381.

Somlyo, A.P., and B. Walz. 1985. Elemental distribution in *Rana pipiens* retinal rods: quantitative electron probe analysis. *J. Physiol. (Camb.)* 358:183–195.

Tamura, T., K. Nakatani, and K.-W. Yau. 1991. Calcium feedback and sensitivity regulation in primate rods. *J. Gen. Physiol.* 98:95–130.

Ungar, F., I. Picopo, J. Letizia, and E. Holtzman. 1984. Uptake of calcium by the endoplasmic reticulum of the frog photoreceptor. *J. Cell Biol.* 98:1645–1655.

Wagner, U., N. Ryba, and R. Uhl. 1989. Calcium regulates the rate of disactivation and the primary amplification step in visual transduction. *FEBS Lett.* 242:249–254.

Yau, K.-W., and K. Nakatani. 1985. Light induced reduction of cytoplasmic free calcium in retinal rod outer segment. *Nature (Lond.)* 313:579–585.

Yau, K.-W., and K. Nakatani. 1988. Calcium and light adaptation in retinal rods and cones. *Nature (Lond.)* 334:69–71.

Yoshikami, S., J.S. George, and W.A. Hagins. 1980. Light-induced calcium fluxes from outer segment layer of vertebrate retinas. *Nature (Lond.)* 286:393–398.