Early receptor current of wild-type and transducin knockout mice: photosensitivity and light-induced Ca^{2+} release

Michael L. Woodruff[1], Janis Lem[2] and Gordon L. Fain[1,3]

[1]*Department of Physiological Science, University of California, Los Angeles, USA*
[2]*Department of Ophthalmology, Program in Genetics, and Tufts Center for Vision Research, Tufts University School of Medicine, Boston, USA*
[3]*Department of Ophthalmology, Jules Stein Eye Institute, University of California, Los Angeles, USA*

We have used suction-electrode recording to measure the early receptor current (ERC) from single, isolated mammalian photoreceptors. When a wild-type mouse rod was illuminated with light sufficient to close all the cGMP-gated channels, a succeeding bright laser flash bleaching a large proportion of the visual pigment produced an ERC, which at 37°C consisted primarily of a single component of transient positive current. The amplitude of total charge movement of this component declined exponentially with successive flashes, consistent with the direct proportionality of the ERC to the quantity of pigment bleached. From the constant of exponential decline, it was possible to estimate the *in vivo* photosensitivity of mouse rhodopsin to be about 6×10^{-9} μm^2 per molecule. We have also measured the ERC from rods of transducin-knockout mice, for which previous illumination to close the cGMP-gated channels was not required. The ERC of these rods was similar to that of wild-type rods but was followed by a slow component of outward current whose maximum amplitude in some cells approached that of the normal light response. This slow current was blocked by L-*cis* diltiazem, indicating that it was produced by ion flux through the cyclic nucleotide-gated channels of the outer segment; however, it could not have been produced by the normal transduction cascade, since it was recorded from rods lacking transducin. Since it was depressed by prior incorporation of the Ca^{2+} buffer BAPTA, it was probably generated by light-activated Ca^{2+} release earlier demonstrated in salamander and zebrafish. Recordings of the ERC from normal and mutant mice may provide a useful tool for the analysis of models of retinal disease, as well as exploration of the molecular origin of light-activated Ca^{2+} release.

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Corresponding author G. L. Fain: Department of Physiological Science, Room 3836, Life Sciences Building, University of California Los Angeles, Los Angeles, CA 90095-1606, USA. Email: gfain@ucla.edu

The early receptor potential (ERP) was first discovered by Brown & Murakami (1964), who were recording from monkey retina with extracellular electrodes and noticed that very bright light produced a rapid change in potential of the same polarity as the a-wave of the electroretinogram but with no detectable latency (see Fain, 2004). Later experiments, particularly by Richard Cone (1964), showed that the ERP increases linearly with the intensity of the stimulus and saturates at about the light level required to bleach all of the photopigment, suggesting that it is caused by the rapid movement of charge across the rod or cone plasma membrane produced by conformational changes in rhodopsin during bleaching.

The ERP has been recorded from single cells in reptiles and amphibians with intracellular recording (Murakami & Pak, 1970; Hodgkin & O'Bryan, 1977). The change in current that produces the ERP, called the early receptor current or ERC, has also been recorded from lower vertebrates with voltage-clamp recording (Hestrin & Korenbrot, 1990; Makino *et al.* 1991; Makino & Dodd, 1996). Although the ERC of human rhodopsin has been studied in an expression system (Shukla & Sullivan, 1999; Brueggemann & Sullivan, 2001), no attempt has been made to record the ERC from an intact photoreceptor of a mammal. Since mouse has become the most useful vertebrate for studying physiological effects of mutations

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in rhodopsin and other transduction proteins, we have attempted to measure the ERC of mouse rods, hoping that recordings of a mammalian ERC might provide a further tool for the analysis of models of retinal degeneration and perhaps also provide a method for relating changes in pigment in a mouse model to changes in the ERP waveform or amplitude recorded from patients in a clinical setting.

We discovered that the ERC of a mouse rod is easily measured and large enough to permit a determination of the photosensitivity of mammalian rhodopsin *in vivo*. When we recorded the ERC from transducin knockout (*Gnat1*$^{-/-}$) animals, we made the surprising observation that the ERC was followed by a current of the same sign as the normal light response, blocked by L-*cis* diltiazem and inhibited by incorporation of the Ca^{2+} buffer BAPTA. These observations appear to provide evidence for a light response in a mammalian photoreceptor independent of the normal transduction cascade and generated by light-activated release of Ca^{2+}, similar to the one previously described for zebrafish cones (Brockerhoff *et al.* 2003).

Methods

Techniques for recording light responses of single mouse rods with suction electrodes have been previously described (Woodruff *et al.* 2002, 2003). In brief, mice kept in darkness for at least 3 hours were killed in dim red illumination by cervical dislocation, according to procedures approved by the Chancellor's Animal Research Committee at UCLA and in conformance with principles regarding the care and use of animals adopted by the American Physiological Society and the Society for Neuroscience. The eye was removed and washed in 1–2 ml of Locke solution, of composition (mM): 140 NaCl, 3.6 KCl, 2.4 $MgCl_2$, 1.2 $CaCl_2$, 3 Hepes, 10 glucose, 5 sodium ascorbate, and 0.02 EDTA at pH 7.4. The retina was isolated and finely chopped under infrared illumination. The suspension of cells was transferred to the recording chamber, where it was perfused at 37°C with Dulbecco's modified Eagle's medium (D-2902, Sigma Chemical, St Louis, MO, USA), supplemented with 15 mM $NaHCO_3$, 2 mM sodium succinate, 0.5 mM sodium glutamate, 2 mM sodium gluconate, and 5 mM NaCl, bubbled with 5% CO_2 in oxygen (pH 7.4). In a few experiments, the cells were perfused at room temperature with an identical solution, except that the $NaHCO_3$ concentration was increased to 23 mM to maintain the pH at 7.4. L-*cis* diltiazem was synthesized and supplied to us by Tocris Cookson (Bristol, UK). BAPTA was incorporated by incubation of isolated cells for 30 min in 50 μM BAPTA-AM (Molecular Probes, Eugene, OR, USA).

Suction pipettes pulled on a Flaming-Brown puller (Sutter Instruments, Novato, CA, USA) and polished on a home-made microforge were filled with Locke solution without glucose or ascorbate. Light stimuli were delivered either with a conventional dual-beam optical bench or from an argon ion laser (American Laser Corporation, Salt Lake City, UT, USA). The intensity of the light was measured with a calibrated photodiode (Graseby Optronics, Orlando, FL, USA). The stimulus from the laser consisted of a 10 μm spot placed in the middle of the outer segment, equidistant from the basal and distal tips. Laser intensities at 488 nm were converted to photons at the wavelength of maximum absorption (λ_{max}) of mouse rhodopsin (507 nm) by multiplying by 0.915 (see Rohrer *et al.* 1999) and are given in the text in units of equivalent photons. Stimulus duration was controlled with electronically driven shutters (Uniblitz, Vincent Associates, Rochester, NY, USA) and calibrated with the same photodiode used for the light bench. Suction-pipette currents were amplified with a patch-clamp amplifier (Warner Instruments Co, Hamden CT, USA), low-pass filtered with an 8 pole Bessel filter (Frequency Devices, Haverhill, MA, USA), acquired with pCLAMP (Axon Instruments, Union City, CA, USA) and a PC computer, and analysed with Quattro Pro (Corel Corporation, Ottawa, Ontario, Canada) and Origin (OriginLab Corporation, Northampton, MA, USA). The frequency of low-pass filtering and the sampling rate were varied in different experiments and are given in the figure legends. Most traces shown in the figures are the averages of many individual responses, and the number of rods and stimulus presentations are also given in the figure legends.

Light micrographs in Fig. 1 were taken with differential interference contrast on a Zeiss IM-35 microscope with a neofluar 63/1.25 NA oil-immersion objective.

Results

Suction-electrode recordings were made from rods completely isolated from other cells. Isolated cells had the advantage that they were easily positioned near the floor of the chamber for stimulation with the argon ion laser. The largest responses were consistently obtained from rods that had intact outer segments and were connected to inner segments by a fine filament of cytoplasm, somewhat variable in length (for examples, see Fig. 1). In most cases the rods lacked nuclei and synaptic terminals. Responses were similar in wave form to those from rods in retinal clumps though generally somewhat smaller in peak amplitude. The tracings in Fig. 2*A* were averaged from 89 cells and show responses to physiological

light intensities. The mean saturating amplitude of the light response from this sample of isolated cells was about 8.9 ± 2.7 pA (mean $\pm$ S.D.), which compares to a mean value of 12.2 pA for a previous sample of cells recorded with the same techniques from retinal clumps (Woodruff *et al.* 2003). Isolated cells were physiologically competent and capable of transducing light into an electrical signal, though we cannot be certain that every feature of the response of these cells is identical to that of rods still attached to retinal clumps.

To record the early receptor current (ERC), we first stimulated a wild-type rod with a 0.5 s flash from the optical bench at an intensity chosen to be bright enough to close all of the cGMP-gated channels but without bleaching a significant fraction of the rhodopsin (Fig. 2*B*). Once the membrane current had reached a steady, saturating value, we gave a brief flash of 488 nm light from the argon ion laser, which delivered 4.8×10^8 equivalent photons μm^{-2}. Using a value for the photosensitivity of 5.7×10^{-9} μm^2 per molecule, calculated below from the data of Fig. 3*C*, we estimate this flash to have bleached 93% of the visual pigment. The suction pipette recorded a brief positive current, which could not have been produced by the cGMP-gated channels, since these had been closed by the previous bench illumination. This current must instead represent the brief displacement current produced by bleaching of rhodopsin. More evidence for this will be given below.

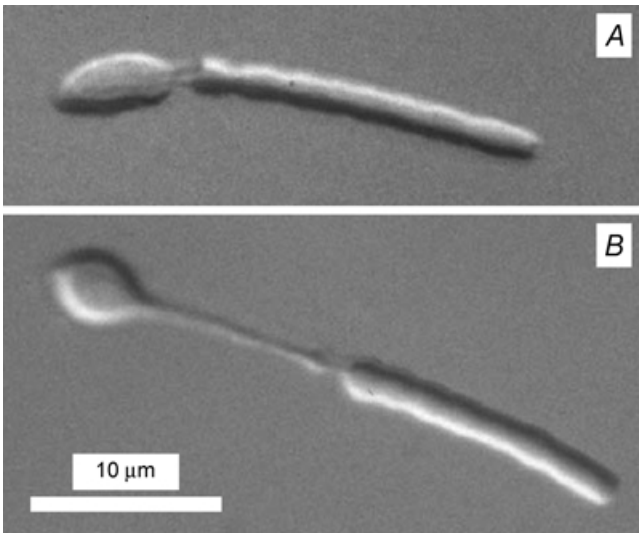

Figure 1. Light micrographs of isolated rods dissociated from the mouse retina (see Methods)
Cells shown in panels *A* and *B* are from the same retina and give examples of morphologies of cells from which recordings were made. Outer segments are shown to the right. Calibration is the same for *A* and *B*.

The wave form of the ERC is shown at a higher temporal resolution in Fig. 2*C*. It is characterized by a large positive excursion, caused by a net movement of charge from the cytosolic side of the membrane toward the extracellular side. This positive component is usually referred to in the earlier literature as R2 (see Fain, 2004). In previous recordings from other species, the R2 component is often

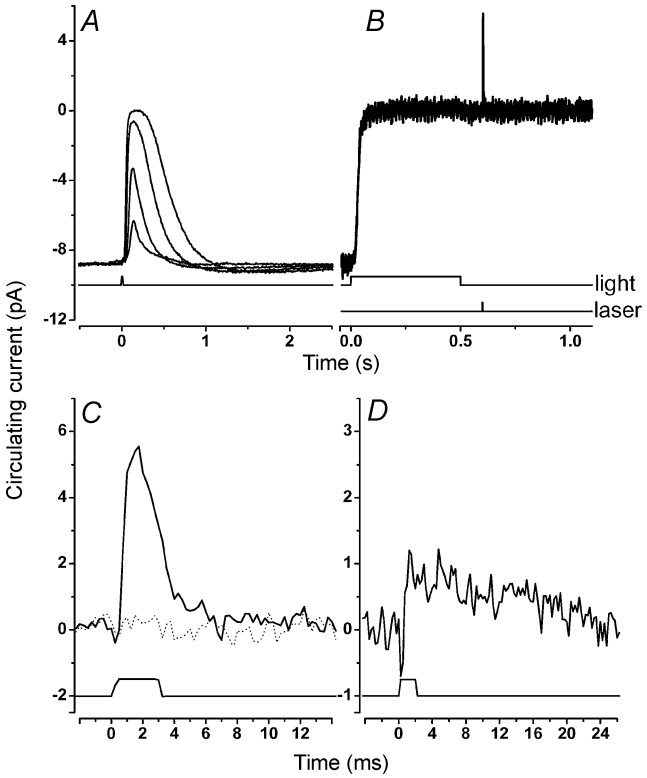

Figure 2. Early receptor current in isolated wild-type mouse rods elicited by bright laser illumination
A, isolated rods were held in a suction electrode placed near the bottom of the recording chamber. Viability and sensitivity of each rod was determined by recording physiological light responses with 20 ms flashes from the optical bench at intensities of 4.5, 11.9, 39.3 and 127 photons μm^{-2} (filtered at $f_c = 50$ Hz with acquisition at 100 Hz). Three to five flashes were given at each intensity, and the traces shown are the mean responses for 89 rods. The mean dark current of these isolated rods was approximately 9 pA. *B*, the same 89 rods were exposed to the halogen source of the optical bench at an intensity 1.67×10^4 photons μm^{-2} s^{-1} for 0.5 s (to close the light-dependent ionic channels) and then to a 2 ms 488 nm argon laser flash at an intensity of 4.8×10^8 equivalent photons μm^{-2}. Data were filtered at $f_c = 1.5$ kHz with acquisition at 4 kHz. The noise of the mean response is greater than in *A* because of the wider bandwidth of the recording. *C*, current from *B* shown on an expanded time scale (continuous line). Dotted line gives response to same laser flash after all the photopigment had been bleached and indicates instrumentation noise at the recording bandwidth. Timing of the laser exposure was measured with a photodiode. *D*, mean ERC from 65 rods as in *C* but recorded at room temperature (approximately 22°C). Note brief negative transient immediately following laser flash. See text.

preceded by a smaller R1 component of opposite polarity (Cone, 1964, 1965; Murakami & Pak, 1970; Hodgkin & O'Bryan, 1977; Hestrin & Korenbrot, 1990; Makino *et al.* 1991; Makino & Dodd, 1996). Although there is a hint of an initial negativity in Fig. 2*C*, it cannot be distinguished from the baseline noise (dotted trace), which is substantial at the large bandwidth of the recording (cut of frequency $f_c = 1500$ Hz; see legend to Fig. 2).

Previous extracellular recordings of ERP from rodent retina at 35–37°C also show only an R2 component, though a negative R1 component has been shown to appear at lower temperature (Cone, 1965; Pak, 1965; see also Brueggemann & Sullivan, 2001). To see if this could also be the explanation for our inability to detect an R1 component, we recorded the ERC from 65 rods at room temperature (approximately 22°C). The averaged ERC from these photoreceptors is shown in Fig. 2*D*. The R2 component of the ERC is smaller in peak amplitude and decays much more slowly; however, the total charge moved during R2 obtained from integrals of the waveforms was similar (14.2 fC for Fig. 2*C* and 12.0 fC for Fig. 2*D*). The R2 component at room temperature was preceded by a brief negative transient, nearly as large in peak amplitude as R2. This may represent an R1 component (see Discussion).

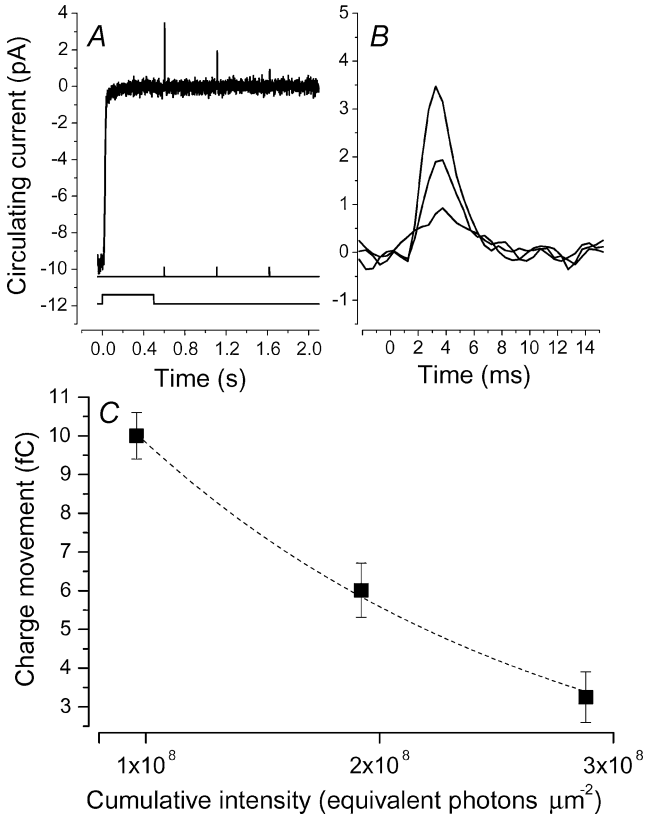

Figure 3. ERC in response to multiple bleaches at the same flash intensity

A, suction-electrode measurements of isolated rods. After recording responses to physiological light intensities as in Fig. 2*A*, each rod was exposed to a saturating illumination for 0.5 s to close the light-dependent channels and then to three successive 2 ms laser flashes of intensity 9.6×10^7 equivalent photons μm^{-2}. The data were filtered at $f_c = 500$ Hz with acquisition at 2 kHz. Trace is average from 64 rods. *B*, currents from the first, second and third laser exposures in *A* superimposed and shown on an expanded time scale. *C*, time integrals of currents from the three laser exposures have been plotted as a function of accumulated photon flux. Data points give mean and S.E.M. from all 64 rods, and the dashed line is a single exponential decay fit to the data.

Photosensitivity of mouse rhodopsin *in vivo*

If the current in response to the bright laser flash is a displacement current produced by molecular movement of rhodopsin, the movement of charge should be strictly proportional to the amount of pigment bleached (Cone, 1964; Hodgkin & O'Bryan, 1977), and the amplitude of the charge moved by a series of successive bleaches of the same intensity should decline exponentially by Beer's Law (see Hestrin & Korenbrot, 1990; Makino *et al.* 1991). An experiment of this kind is given in Fig. 3. An initial stimulus was given from the light bench to close the cGMP-gated channels as in Fig. 2*B*, and this was then followed by a series of three flashes of intensity 9.6×10^7 equivalent photons μm^{-2}, each estimated to bleach 42% of the remaining rhodopsin. All recordings were made at 37°C. The R2 responses to the flashes are shown at higher temporal resolution in Fig. 3*B*; each trace is the average of 64 rods.

The R2 responses from each of the rods for the experiment of Fig. 3 were individually integrated from the beginning of the flash over a period of 8 ms to calculate the charge movement. The mean and S.E.M. values of these measurements have been plotted against cumulative intensity in Fig. 3*C*. Note that, since each of the flashes was of the same intensity, plotting cumulative intensity is equivalent to plotting flash number. The means could be fitted with single exponential decay, consistent with the simple proportionality of the charge movement to the number of rhodopsin molecules bleached.

The decay of the amplitude of charge movement to a series of flashes of the same intensity can be used to estimate the *in vivo* photosensitivity of mouse rhodopsin (Makino *et al.* 1991; Makino & Dodd, 1996). The amplitude of the charge moved during the ERC as a function of the cumulative light intensity should decrease according to:

$$Q_{\mathrm{ERC}} = A \exp(-P I_t) \qquad (1)$$

where Q_{ERC} is the charge moved during the ERC in response to an individual flash, P is the photosensitivity, I_t is the cumulative light exposure, and A is the product of a series of constants: Q_{100}, the charge moved for a 100% bleach; a factor $\{\exp(PI) - 1\}$, where I is the intensity of a single flash in the flash series; and F, the fraction of outer segment current collected by the suction electrode (see Makino *et al.* 1991). The best-fitting values for the data in Fig. 3*C* give an A of 17.3 and a P of 5.7×10^{-9} μm^2 per molecule. The factor $\{\exp(PI) - 1\}$ can be calculated to be equal to 0.71, and since F may be of the order of 0.5 (Baylor & Nunn, 1986), Q_{100} can be estimated to be about 50 fC.

ERC of rods from transducin knockout mice

In rods from *Gnat1*$^{-/-}$ mice that lack the G protein transducin, there are no responses to physiological light levels since the visual cascade cannot be activated (Calvert *et al.* 2000). It should therefore be possible to record the ERC without prior illumination, since the only current expected from the photoreceptor is the displacement current produced by the conformational change in rhodopsin. Recordings of the ERC in transducin knockout mice are shown in Fig. 4. The records in *A* and *B* are the same response averaged from 40 rods to the same laser flash used in Fig. 2, calculated to bleach 93% of the rhodopsin. In *A* the recordings have been low-pass filtered at 1500 Hz, and in *B*, at 35 Hz (note difference in scale for the ordinate). The ERC in Fig. 4*A* is similar in amplitude to that recorded from normal mice but has a somewhat accelerated wave form (inset). The reason for the difference is not known but may be the result of the less negative membrane potential in the transducin knockout animals, for which the cGMP-gated channels would be expected at least initially to remain open even in light. The acceleration of kinetics may also at least in part arise from a difference in the time constant of the rods, which would be expected to be shorter in the transducin knockout animals.

We were surprised to discover that in addition to the ERC there was a subsequent, slower component of response produced by the laser flash, which was more easily visualized after low-pass filtering at lower frequency as in Fig. 4*B*. In contrast to the ERC, this slower current lasted for over a second and resembled the response produced by light-activated Ca^{2+} release previously recorded from zebrafish *nof* cones, which lack cone transducin (Brockerhoff *et al.* 2003). Although the response averaged about 0.5 pA from a sample of many rods, in some cells this slow current was much larger. Figure 4*C* shows the mean response of three rods giving slow currents of peak amplitude 5–7 pA. At this current scale (and after filtering at 35 Hz), the ERC itself was barely detectable.

If, as in zebrafish cones, this slow current is produced by closing of the cGMP-gated channels in response to a light-activated increase in outer segment Ca^{2+} concentration, we should be able to block it both with the cGMP-gated channel blocker L-*cis* diltiazem (see Kaupp & Seifert, 2002) and by prior incorporation of the Ca^{2+} buffer BAPTA. Experiments of this kind are given in Fig. 5. Panel *A* gives

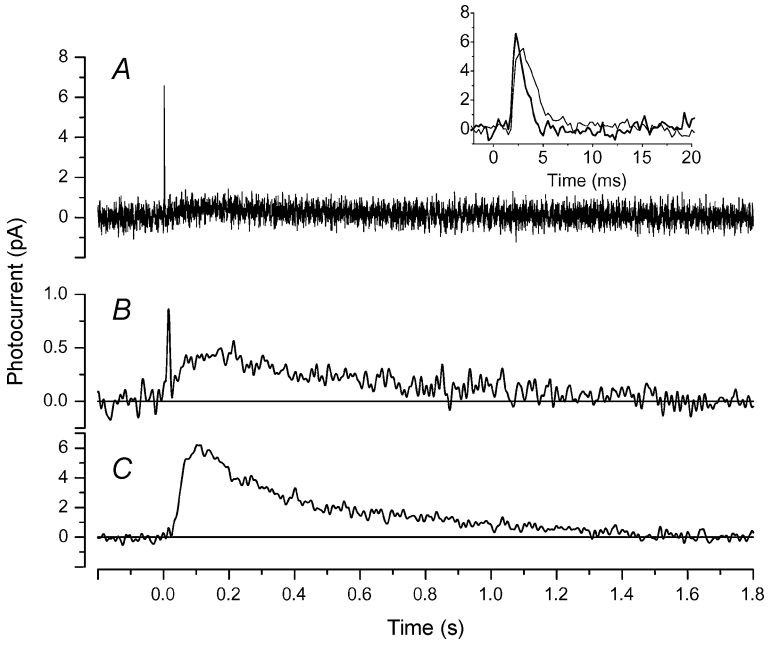

Figure 4. Response to laser illumination in rods of *Gnat1*$^{-/-}$ mice
A, isolated rods from *Gnat1*$^{-/-}$ mice were stimulated as in Fig. 2*B* except that no light from the optical bench was given to close outer segment channels. Trace is mean of 40 rods. Data were filtered at $f_c = 1.5$ kHz with acquisition at 4 kHz. Note small positive hump of current following ERC. A second flash given to the rods (not shown) gave neither ERC nor positive current hump. Inset compares wave form of ERC at higher temporal resolution for wild-type rods from Fig. 2 (thin line, 89 rods) and *Gnat1*$^{-/-}$ rods (thick line, 40 rods). See text. *B*, individual currents from the 40 rods in *A* were individually filtered electronically at 35 Hz, averaged and plotted on an expanded ordinate to show more clearly the slow component of current. *C*, average of three rods giving slow currents 5–7 pA in amplitude. Approximately 5% of rods recorded showed such large responses.

the average of the responses of 65 *Gnat1*$^{-/-}$ rods in control solution and without BAPTA incorporation. The rods used for this average were different from those for Fig. 4*A* and *B* and were from the same retinas used for the recordings of parts *B* and *C* of Fig. 5. The records in panels *B* and *C* show the average of the responses to the laser flash of 26 rods perfused with 100 μM L-*cis* diltiazem, and of 38 rods after prior incorporation of 50 μM BAPTA-AM. Both treatments inhibited the slow current, suggesting that this component of the response is generated by a process that is independent of the normal transduction cascade, and which produces a change in outer segment Ca^{2+} concentration that reduces the current through the cGMP-gated channels.

Discussion

We have shown that the wave form of the ERC in mouse is similar to that previously recorded from single rods and cones in lower vertebrates, consisting of a large and rapid component of outward current, usually referred to as R2. In other species the ERC has been shown to have an additional smaller component of inward current called R1, which may be obscured at 37°C by the molecular transition that produces R2 (see Cone, 1965, 1967; Pak, 1965). We tested this possibility by recording the ERC at room temperature and succeeded in detecting a brief negative transient preceding the R2 component (Fig. 2*D*). We think this may represent R1, though the noise at the bandwidth of our recording was not sufficiently small even after extensive averaging to exclude other explanations. If this negative transient is the R1 component, its amplitude may be underestimated, since the kinetics of the ERC in a suction-electrode recording may be limited by the electrical properties of the cell, i.e. the membrane time constant and internal resistivity.

We have shown that the amplitude of total charge movement during the ERC declined exponentially with successive flashes (see Fig. 3*C*), consistent with the direct proportionality of the ERC to the quantity of pigment bleached. We have used the amplitude of the charge movement for a series of successive flashes to estimate the value of the photosensitivity of mammalian rod rhodopsin. Our estimate of 5.7×10^{-9} μm^2 per molecule is somewhat smaller than the value of about 10^{-8} μm^2 per molecule previously obtained for A_1-based rhodopsin in solution (see Dartnall, 1968). Although part of this difference is probably due to orientation of visual pigment in the plasma membrane of the rod (see Makino *et al.* 1991), the greater part may be the result of light scatter by the suction recording pipette. Since the outer segment has been drawn into the pipette, light must pass through the glass before it reaches the rhodopsin molecules, and some of the incident illumination will be lost by scattering. An overestimate of I_t in eqn (1) would produce an underestimate of P. The value of P we have measured is nevertheless quite useful, since after correction for the difference in orientation of rhodopsin in the plasma and disk membranes, it can be used to calculate with some accuracy the fraction of pigment bleached in the outer segment during a suction-electrode experiment.

The laser spot in our measurements was placed so that it was unlikely to have stimulated either basal disks or the tip of the outer segment (see Methods). The

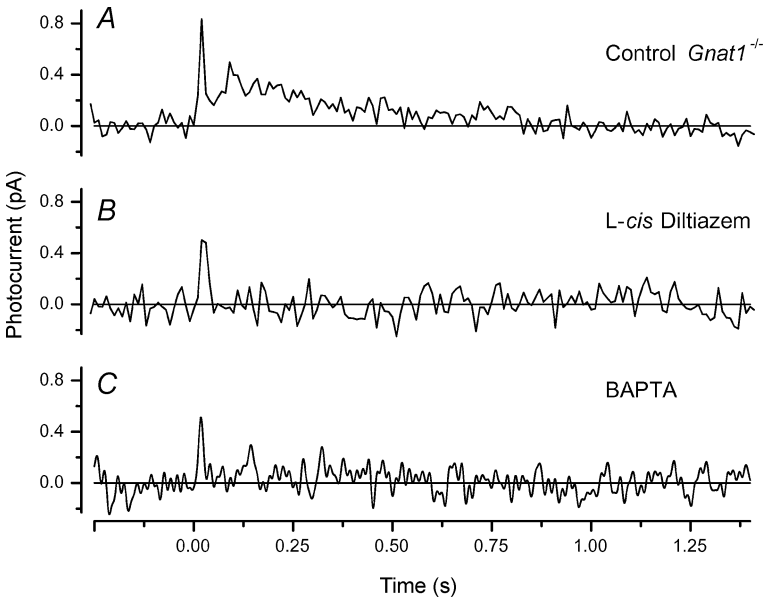

Figure 5. Laser-induced slow outward current in rods of *Gnat1*$^{-/-}$ mice is blocked by L-*cis* diltiazem and by prior incorporation of the Ca^{2+} buffer BAPTA
A, untreated control rods from *Gnat1*$^{-/-}$ mice were recorded at either $f_c = 35$ Hz with acquisition at 100 Hz (in the diltiazem experiment) or electronically filtered at 35 Hz after initial filtering at $f_c = 1.5$ kHz with acquisition at 4 kHz (in the BAPTA experiment). The mean of 65 untreated control rods for the two experiments is shown. *B*, the mean of 26 rods perfused with Dulbecco's modified Eagle's medium containing 100 μM L-*cis* diltiazem. *C*, the mean of 38 rods pre-incubated with 100 μM BAPTA-AM for 30 min (see Methods). The individual currents were electronically filtered at $f_c = 35$ Hz prior to averaging.

membrane surface contributing to the ERC was therefore likely to have been only the surface membrane, which for a 10 μm diameter spot and a rod 1.2 μm in diameter can be calculated to have an area of about 38 μm^2. On the assumption that the density of rhodopsin molecules in the surface membrane is the same as in the disk, and that the disk density is 2.7×10^4 molecules μm^{-2} (see Fein & Szuts, 1982), we estimate from the total charge movement of about 50 fC that the bleaching of a single mouse rhodopsin molecule causes the movement of 0.3 of an electronic charge perpendicular to the surface of the plasma membrane. If the density of pigment molecules in the surface membrane is half that in the disk (Molday & Molday, 1987), this would rise to 0.6 of a charge per molecule. These estimates are uncertain, since the fraction of current collected by the suction pipette may have been greater or less than our estimate of 0.5, but they appear to be somewhat larger than the values for charge movement per molecule estimated from ERP and ERC measurements in other species (0.08–0.2, see Hodgkin & O'Bryan, 1977; Hochstrate *et al.* 1982; Hestrin & Korenbrot, 1990; Makino *et al.* 1991), perhaps indicating a difference between mammalian rhodopsin and the pigment of lower vertebrates.

In rods of *Gnat1*$^{-/-}$ mice, the ERC was followed by a slow component of outward current of the same sign as the photocurrent. This response must arise from the closing of cGMP-gated channels, since it can be blocked by L-*cis* diltiazem (Fig. 5*B*). Although the mean value of this current was only about 0.5 pA, in some rods it was of the order of 5–7 pA, nearly as large as the maximum value of the normal light response. Although such large responses were recorded from only a small minority of the photoreceptors, we think it unlikely that they were produced by the normal transduction cascade from a few anomalous rods having an intact response (see Calvert *et al.* 2000), or from cones, which in a *Gnat1*$^{-/-}$ mouse would have had functional tranducin. The response in Fig. 4*C* rises much more slowly and decays much more rapidly than would be expected for channel closing produced by the normal transduction cascade in either rods or cones for such bright flash intensities.

Since the slow current response can also be blocked by incorporation of the Ca^{2+} buffer BAPTA (Fig. 5), it is probably produced by light-activated Ca^{2+} release. Previous experiments have shown that light-activated release requires bright light (Matthews & Fain, 2001, 2003). Furthermore, in *nof* mutant zebrafish cones that lack transducin bright light can produce an electrical response (Brockerhoff *et al.* 2003) similar to the one we have recorded from transducin knockout mouse rods. In *nof* zebrafish cones this response also rises and decays much more slowly than the normal light response, and it is also blocked by diltiazem and by incorporation of the Ca^{2+} buffer BAPTA. Although it is not presently possible to record light-activated Ca^{2+} release from mouse rods directly, the similarity of the properties of the response in mouse rods to that in zebrafish cones argues strongly that both are produced by a similar phenomenon.

Since the recording of the ERC from mouse rods is relatively straight forward, it may be possible to use this method in a variety of experiments on isolated wild-type and mutant photoreceptors. The ERC could, for example, be used to estimate rhodopsin concentration during vitamin A deprivation, or in mutant animals such as *Rpe65*$^{-/-}$ mice (Woodruff *et al.* 2003), which have a reduced rhodopsin content. It might also provide a useful measure of rhodopsin expression in animals for which the normal light response could not be measured, for example in animals for which the cGMP-gated channels or guanylyl cyclase were mutated.

If the slow current we record in transducin knockout rods is indeed produced by light-activated Ca^{2+} release, the recording of this current in mutant animals might facilitate an investigation of the molecular mechanism of Ca^{2+} release. It is at present unknown whence the Ca^{2+} originates or how it produces an electrical response. Measurements from mice lacking transducin but also lacking other proteins, such as Ca^{2+} binding proteins or components of IP_3-gated Ca^{2+} release, might make it possible to explore the origin of this interesting phenomenon in greater detail. Although light-induced Ca^{2+} release is unlikely to produce a significant change in the outer segment Ca^{2+} concentration at physiological light levels (Matthews & Fain, 2003), it may reflect light-dependent changes in Ca^{2+} buffering or other phenomena of importance to the function or homeostasis of the photoreceptor.

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