Spontaneous Activation of Light-sensitive Channels in *Drosophila* Photoreceptors

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ABSTRACT In *Drosophila* photoreceptors light induces phosphoinositide hydrolysis and activation of Ca^{2+}-permeable plasma membrane channels, one class of which is believed to be encoded by the *trp* gene. We have investigated the properties of the light-sensitive channels under conditions where they are activated independently of the transduction cascade. Whole-cell voltage clamp recordings were made from photoreceptors in a preparation of dissociated *Drosophila* ommatidia. Within a few minutes of establishing the whole-cell configuration, there is a massive spontaneous activation of cation-permeable channels. When clamped near resting potential, this "rundown current" (RDC) accelerates over several seconds, peaks, and then relaxes to a steady-state which lasts indefinitely (many minutes). The RDC is invariably associated with a reduction in sensitivity to light by at least 100-fold. The RDC has a similar absolute magnitude, reversal potential, and voltage dependence to the light-induced current, suggesting that it is mediated by the same channels. The RDC is almost completely ($\geq 98\%$) blocked by La^{3+} (10–20 μM) and is absent, or reduced and altered in the *trp* mutant (which lacks a La^{3+}-sensitive light-dependent Ca^{2+} channel), suggesting that it is largely mediated by the *trp*-dependent channels. Power spectra of the steady-state noise in the RDC can be fitted by simple Lorentzian functions consistent with random channel openings. The variance/mean ratio of the RDC noise suggests the underlying events (channels) have conductances of ~1.5–4.5 pS in wild-type (WT), but 12–30 pS in *trp* photoreceptors. Nevertheless, the power spectra of RDC noise in WT and *trp* are indistinguishable, in both cases being fitted by the sum of two Lorentzians with a major time constant (effective "mean channel open time") of 1–2 ms and a minor component at higher frequencies (~0.2 ms). This implies that the noise in the WT RDC may actually be dominated by non-*trp*-dependent channels and that the *trp*-dependent channels may be of even lower unit conductance.

INTRODUCTION

Substantial evidence indicates that excitation in invertebrate rhabdomeric photoreceptors is mediated at least in part by the ubiquitous phosphoinositide cascade (Brown, Rubin, Ghalayini, Tarver, Irvine, Berridge, and Anderson, 1984; Fein,

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Payne, Corson, Berridge, and Irvine, 1984; Devary, Heichal, Blumenfeld, Cassel, Suss, Barash, Rubinstein, Minke, and Selinger, 1987; Bloomquist, Shortridge, Schneuwly, Pedrew, Montell, Steller, Rubin, and Pak, 1988; revs; Payne, Walz, Levy, and Fein, 1988; Nagy, 1991; Minke and Selinger, 1991). However, the final pathway of excitation, linking phosphoinositide hydrolysis and Ca^{2+} release to the activation of cation channels in the plasma membrane, has remained surprisingly elusive. One reason for the uncertainty is suggested by recent evidence indicating that there are at least two functional classes of light-sensitive channel (*Lima,* Nasi, 1991; *Limulus,* Deckert, Nagy, Helrich, and Stieve, 1992; *Drosophila,* Hardie and Minke, 1992; *Pecten,* Nasi, and del Pilar Gomez, 1992). This raises the possibility that they may be activated by different mechanisms. In *Drosophila,* at least, any divergence would appear to be subsequent to inositol 1,4,5 trisphosphate ($InsP_3$) production, since all responsiveness to light is abolished by mutations in the *norpA* gene (Pak, Grossfield, and White, 1969; Minke and Selinger, 1992), which encodes a light-dependent phospholipase C (Yoshioka, Inoue, and Hotta, 1985; Bloomquist et al., 1988; Toyoshima, Matsumoto, Wang, Inoue, Yoshioka, Hotta, and Osawa, 1990). Our own recent results indicate that one class of light-sensitive channel in *Drosophila* has a high selectivity for Ca^{2+} ions, is blocked by La^{3+} and is absent in the transient receptor potential (*trp*) mutant (Hardie and Minke, 1992). The light-sensitive channels remaining in the *trp* mutant have a much reduced permeability to Ca^{2+}, with an ionic selectivity profile more indicative of a non-specific cation channel (Hardie and Minke, 1992). The sequence of the recently cloned *trp* gene (Montell and Rubin, 1989; Wong, Schaefer, Roop, La Mendola, Johnson-Seaton, and Shao, 1989) shows homologies with vertebrate voltage-gated Ca^{2+} channels (dihydropyridine receptors) (Phillips, Bull, and Kelly, 1992); however, it includes only one of the four putative transmembrane domains suggesting that it encodes just one subunit of a tetrameric channel. A closely homologous gene (*trpl*) also expressed in *Drosophila* photoreceptors might encode either another class of light-sensitive channel (Phillips et al., 1992), or a second subunit of the same channel. These results suggest that the *trp* and *trpl* gene products could define a new family of channel proteins responsible for phosphoinositide-mediated Ca^{2+} entry (Hardie and Minke, 1992). Very few electrophysiological studies of such Ca^{2+} entry channels exist (but see Lückhoff and Clapham, 1992; Hoth and Penner, 1992, 1993), and the mechanism(s) responsible for their activation represents one of the most controversial areas of cellular Ca^{2+} homeostasis (reviews: Irvine, 1991, 1992; Putney, 1990, 1992; Berridge, 1993).

Although some properties of the light-induced current (LIC) in *Drosophila* have recently been characterized (Hardie, 1991*a;* Ranganathan, Harris, Stevens, and Zuker, 1991; Hardie and Minke, 1992), very little is known of the properties of the underlying channels. One reason is that it has not proved possible to access single light-sensitive channels with patch electrodes in *Drosophila.* An alternative is to use noise and kinetic analysis of whole-cell recordings of the LIC. Normally this approach would be hampered since the LIC is dominated by the noise arising from the summation of single photon responses (quantum bumps). In this study, we report that during whole-cell recording from dissociated ommatidia there is a spontaneous activation of cation channels in the plasma membrane. We provide evidence that this "rundown current" (RDC) is mediated by the light-sensitive channels themselves,

uncoupled from the transduction machinery. Two functional classes of channels can be at least partially dissected via mutation (*trp*) or pharmacological intervention (La^{3+}). By determining current–voltage relationships and performing noise analysis (Colqhoun and Hawkes, 1977), we provide a preliminary description of channel properties including estimates of their effective conductance, open time, and voltage dependence.

MATERIALS AND METHODS

Flies

The wild-type (WT) strain was Oregon R; for most experiments we used white-eyed (*w*) flies lacking all retinal screening pigments. No significant difference has been detected between these and flies with normal pigmentation. The following null mutants (all on a *w* background) were also used: trp^{CM} (Cosens and Manning, 1969; Montell and Rubin, 1989) lacking a light-dependent Ca^{2+} channel (Hardie and Minke, 1992), and two strains both lacking the rhodopsin gene *ninaE* (O'Tousa, Baehr, Martin, Hirsh, Pak, and Applebury, 1985; Zuker, Cowman, and Rubin, 1985): *sr Df(3R) ol17* e^s and $ninaE^{ora}$ $e^s/TM6$. Flies were raised at 25°C. Late-stage pupae (p15) staged according to Bainbridge and Bownes (1981) or newly eclosed adults were used.

Whole-Cell Recordings

Dissociated ommatidia were prepared as previously described (Hardie, 1991*a,b;* Hardie and Minke, 1992) using mechanical trituration without enzymatic treatment. Whole-cell recordings were made at room temperature (19–21°C) using boro-silicate electrodes with resistances ~5 MΩ. All recordings were from photoreceptors R1–6 (Hardie, 1985). Series resistances (R_s) were in the range 7–20 MΩ, and 65–85% compensation was routinely used (except for noise measurements—see below). The resulting R_s errors (voltage error in mV = R_s × current) were calculated and corrected for, and in any case were <5 mV for all quantitative data presented in this study. Data were also corrected for junction potentials (see subsection *Solutions* below).

Illumination was via a 50-W halogen lamp, filtered by Wratten ND filters (Eastman Kodak Co., Rochester, NY), and an OG 530 yellow filter, and was delivered to the preparation by a lightguide positioned 2 cm over the bath. Log −6.0 attenuation corresponds to ~5–10 effectively absorbed photons per second (determined by direct counting of quantum bumps).

Noise Analysis

For noise analysis data were recorded without any series compensation. The current signal was filtered at 2 kHz (four-pole Bessel) and a number of records, each of 2,048 points, sampled at 5 kHz. To correct for any slight change in current during this sampling period (maximally ~20% change during dynamic phase of the RDC and <5% for the steady-state used for spectral analysis), a linear regression was calculated and subtracted from each record. The power spectrum of the steady-state noise was obtained by taking the ensemble average of spectra extracted by fast Fourier transform from a number (12–48) of nonoverlapping 512-point segments of the records. The power spectra were corrected for instrumentation and recording noise by subtracting the power spectrum of noise recorded in darkness before the rundown current developed. Spectra were corrected for the filtering effect of the clamp time constant (determined from the decaying phase of the capacitive transients—range 0.3–0.9 ms) and fitted with Lorentzian functions using a Marquardt curve fitting routine (P. Fit). To minimise the filtering effect of the clamp time constant, null mutants of the rhodopsin gene

ninaE were often used. RDCs in *ninaE* were similar to WT, but since this mutant produces a much reduced number of microvilli, the capacitance (C) of the cells (and hence the clamp time constant, $R_s \cdot C$) is considerably lower than in WT.

Estimates of the amplitude of the underlying single channel events were made from the ratio of variance to mean (Colquhoun and Hawkes, 1977), and single-channel conductances calculated as chord conductances after correcting for the series resistance error and assuming the reversal potential estimated for the RDC. A significant proportion (typically 25–50%) of the variance can be expected to be filtered by the clamp time constant. This was corrected for by a factor obtained by comparing the areas under theoretical power spectra (based on the Lorentzian fits to the data) before and after convolving with the RC filtering function of the measured clamp time constant ($R_s \cdot C$):

$$1/[1 + (2\pi \cdot f \cdot R_s \cdot C)^2] \quad (1)$$

where f = frequency.

Solutions

Bath Ringer contained (in mM): 120 NaCl, 5 KCl, 8 $MgSO_4$, 10 *N*-tris-(hydroxymethyl)-methyl-2-amino-ethanesulfonic acid (TES), 25 sucrose, 5 proline. $CaCl_2$ was added as indicated. When it was necessary to block K channel activity, pipette solution contained (in mM) 120 CsCl 15 tetraethyl ammonium (TEA) Cl, 2 $MgSO_4$, 10 TES (junction potential −3 mV). Unless otherwise stated EGTA was either included at very low (100 μM) concentrations or not added at all. For some experiments pipettes contained 135 mM K gluconate (junction potential −10 mV) or KCl (junction potential −2 mV) instead of CsCl and TEACl.

RESULTS

Rundown Is Associated with Spontaneous Channel Activation

WT. As previously reported (Hardie, 1991*b*), unless special precautions are taken (Hardie et al., manuscript in preparation), the LIC in *Drosophila* photoreceptors runs down within a few minutes of establishing the whole-cell recording configuration. When the cells are clamped near resting potential (~ −60 mV), the rundown is associated with the development of a conspicuous inward current (RDC). Although the RDC will always develop spontaneously in the absence of stimulation, it can also be directly induced by bright illumination. The onset of spontaneous rundown is rather variable, occurring typically within 5–7 min, but sometimes within 1–2 min of establishing the whole-cell configuration. This is to some extent related to the access (series) resistance of the patch-pipette, but also seems to vary from one preparation to another. Rundown occurs with a variety of basic intracellular pipette solutions including ones based on CsCl, CsCl and TEA Cl, KCl, and K gluconate and is not prevented by inclusion of ATP (4 mM) in the recording pipette or Ca^{2+} buffering with 5–10 mM EGTA (pCa 7–8). Conditions which prevent rundown will be discussed in a later paper addressing possible molecular mechanisms of channel activation (Hardie et al., manuscript in preparation). Spontaneous rundown currents follow a characteristic time course (Fig. 1 *A*), accelerating over a period of several seconds, reaching a peak which can be at least 1 nA in amplitude. Invariably, this phase is associated with an equally rapid decline in the sensitivity of the cell to light (e.g., Fig.

1 *C*). After peaking, this inward current relaxes to a steady state which lasts indefinitely (many minutes) and is associated with conspicuous high frequency noise.

trp. In the *trp* mutant, which lacks one class of Ca^{2+} selective channel (Hardie and Minke, 1992), significant rundown currents often fail to develop at all (22/34 cells monitored for rundown), in which case rundown is simply represented by a loss of responsiveness to light. If rundown currents do develop however, they reveal

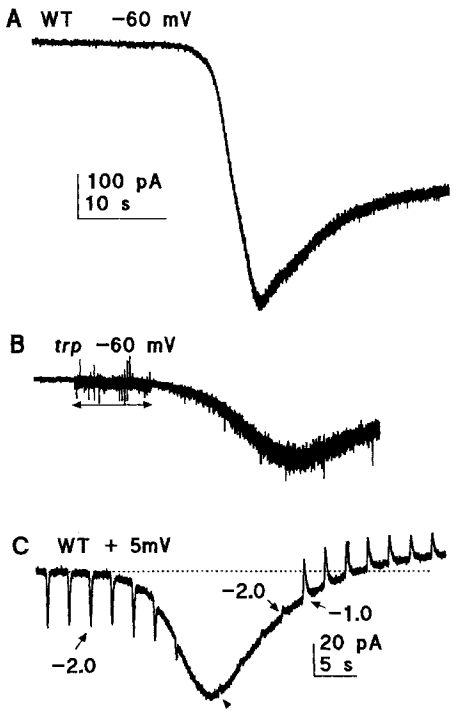

FIGURE 1. Spontaneous RDCs recorded a few minutes after establishing whole-cell recording configuration. (*A*) Cell from adult wild-type (*WT*) photoreceptor, clamped at resting potential (−60 mV); bath Ca^{2+} 0.5 mM, electrode K-gluconate. Notice the characteristic waveform and the increase in high frequency noise. (*B*) RDC recorded in adult *trp* at −60 mV, 0.5 mM Ca^{2+} Ringer (same scale); in the interval indicated by the arrow, series resistance was checked with 10-mV voltage steps. The RDC is smaller than in WT and develops more slowly, and the bandwidth of the associated noise is greater. (*C*) WT photoreceptor (adult) clamped at +5 mV (~2 mV below reversal potential of the light response in this cell). Bath Ringer 0.25 mM Ca^{2+}, electrode Cs/TEA Cl. 10-ms flashes of light are repeated at 3-s intervals, intensity log −2.0, increased to log −1.0 after development of RDC (*arrows*). Both the RDC and the light induced currents are initially inward but then reverse in sign during rundown. The LIC reverses immediately after the RDC has reached its maximum inward excursion (*arrowhead*); the RDC may reverse at the same time; however, this cannot be distinguished from a simple relaxation in the absolute conductance; at the latest the RDC has reversed by the time it crosses the resting baseline (*dotted line*). After rundown, the reversal potential has shifted by ~10–15 mV so that the driving force ($E_M - E_{rev}$ for the outwardly directed LICs (~+8–13 mV) at the end of the trace is considerably greater than at the start (~−2 mV).

significant differences from WT (c.f. Fig. 1, *A* and *B*). Firstly, the absolute magnitude of the RDC was much smaller in *trp;* under similar conditions (adult flies, 0.5 mM external Ca^{2+}, −60 mV) peak rundown currents recorded in *trp* averaged 258 ± 80 pA, $n = 8$; c.f 650 ± 107 pA, $n = 10$ for WT). Secondly, the associated high frequency noise was more conspicuous (see below). Finally the RDC in *trp* develops more gradually and is not indefinitely maintained but decays to near baseline after 1

or 2 min (not shown). Further differences in voltage dependence and noise characteristics are described below.

Ionic Selectivity and Voltage Dependence of the RDC

WT. The induction by bright light, strict correlation with the decline in sensitivity to light, and alteration by the *trp* mutation suggest that the RDC might represent spontaneous activation of at least a subpopulation of the light-sensitive channels. This possibility was tested more rigorously by comparing the reversal potential, voltage dependence, and pharmacology of the RDC and the LIC.

To compare the reversal potential (E_{rev}) of the rundown current and the light-induced current, cells were clamped within a few millivolts (<5 mV) of E_{rev} of the LIC so that small inward or outward currents could still be recorded in response to light flashes (e.g., Fig. 1 *C*). Under these conditions the RDC always developed with the same polarity (inward, $n = 5$ cells; or outward, $n = 3$ cells) as the light responses. When the cells were clamped just below E_{rev}, after a few seconds the initially inward RDC reverses in sign, becoming outward (Fig. 1 *C*). Although the exact point at which the RDC reverses cannot be determined in Fig. 1 *C* (as the RDC presumably also relaxes during the recording), it is clear that the residual light responses also reverse their polarity over the same time period. In other words, not only are E_{rev} of the LIC and RDC very similar, but they also show a similar shift during rundown. Direct confirmation that the current is carried by cations was shown by substituting external cations with *N*-methyl-D-glucamine, which does not permeate the light-sensitive channels (Hardie and Minke, 1992) after rundown had developed. Any inward rundown current is effectively abolished under these conditions (data not shown).

The current–voltage (i-V) relationship of the photoreceptors was determined by applying 50-ms voltage pulses in the range +80 to −100 mV in 20-mV steps (Fig. 2). Before rundown, photoreceptors have rather high input resistances, typically of the order of 1 GΩ, and the membrane is approximately ohmic when measurements are made using an internal solution consisting of CsCl and TEA Cl to block K channel activity (Fig. 2 *A*). However, below ~ −80 mV a slowly developing inward rectification is usually observed. This is probably mediated by chloride channels since it is absent if internal chloride is substituted with gluconate. The origin of the more rapid transient inward rectification and the slight outward rectification observed at very depolarized potentials) is unclear, although both are consistently observed in WT and *trp* photoreceptors. Possibly it might represent residual contribution of voltage-sensitive K channels, which would generate currents in excess of 10 nA in the presence of internal potassium (Hardie, 1991*b;* W. Hevers and R. Hardie unpublished data). After rundown there is a dramatic change in the i-V relationship (Fig. 2, *B* and *D*). The input resistance drops to 10–100 MΩ and a marked inward and outward rectification develops similar to that observed for the LIC (Hardie, 1991*a;* see also Fig. 4). Current responses to the instantaneous voltage steps also show characteristic transient changes which are analysed in more detail in a companion paper (Hardie and Minke, 1994). The absolute magnitude of the current can reach values >10 nA (at ±80 mV) which is similar to the maximum light-induced current (Ranganathan et al., 1991; Hardie, unpublished data). Subtracting sets of i-V traces (as in Fig. 2 *D*) before and after rundown provides an alternative means of estimating

E_{rev} of the RDC (+3.8 ± 3.7 mV [n = 9] at 2 mM Ca; values corrected for −3-mV junction potential). Although this is some 10 mV negative to E_{rev} of the LIC before rundown, it is indistinguishable from E_{rev} of the residual LIC after rundown (see below; c.f. Figs. 2 *D* and 4 *C*).

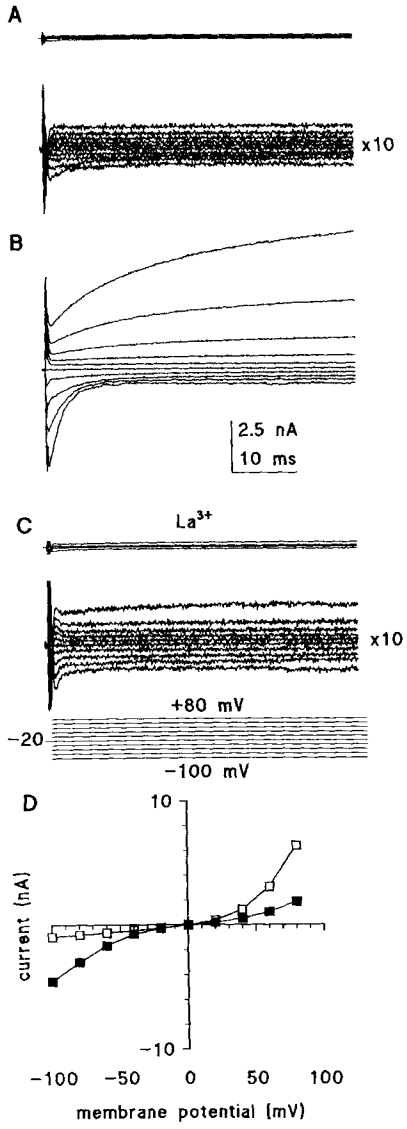

FIGURE 2. Current–voltage relationship of WT photoreceptors: before and after development of RDC, and after block of RDC by La^{3+}. (*A* and *B*) currents elicited in response to 50-ms voltage steps (between +80 and −100 mV at 20-mV intervals) from a holding potential of −20 mV, before and after rundown in 0.5 mM Ca^{2+} Ringer using Cs/TEA Cl–based electrode solution. No leak subtraction has been applied to any of the traces. (*A*) Before rundown only small largely linear leak currents are observed, although on a 10× expanded scale some rectification can be observed. (*B*) After rundown large transient rectifying currents are observed. (*C*) 2 min after adding 20 μM La^{3+} to the bath (otherwise identical Ringer). The RDC is virtually abolished (after subtraction of the control i-V data set in *A;* it can be estimated that 98% of the RDC has been eliminated). (*D*) i-V relationship of the RDC from traces in *B* determined from the instantaneous currents (1 ms after the voltage step; ■) and the steady-state (average current between 25 and 50 ms after voltage step; □). The currents before rundown in *A* were subtracted before measurement. Data not corrected for series resistance errors (maximally +10 mV at −100 mV) or junction potential errors. All traces from the same adult photoreceptor.

To address the question of which of the two suspected classes of light-sensitive channel is responsible for the RDC in WT flies, we tested the effect of La^{3+} which specifically blocks the *trp*-dependent channels (Hochstrate, 1989; Suss-Toby, Selinger, and Minke, 1991; Hardie and Minke, 1992). If micromolar concentrations

of La^{3+} are applied to the bath after development of rundown, the RDC is rapidly (1–2 min) and almost completely blocked (≥ 98%) suggesting that it is largely carried by the *trp*-dependent channels (Fig. 2 *C*).

trp. Similar voltage protocols applied to *trp* photoreceptors after rundown reveal significant differences (Fig. 3). The inward rectification is much less apparent, and although there is still a substantial outward rectification, this develops more rapidly. Single exponentials fitted to the currents at +80 mV yield time constants of 4.3 ± 1.0 ms (n = 5) in *trp* compared with 10.8 ± 3.3 ms (n = 5) in WT. Currents induced by depolarizing steps also show characteristic noisy fluctuations which are not apparent in the WT traces.

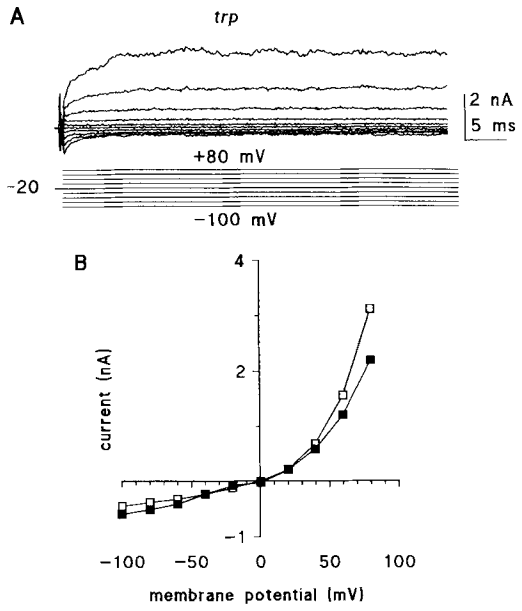

FIGURE 3. Current-voltage relationship of RDC recorded in *trp*. (*A*) Currents elicited in response to 50-ms voltage steps made after rundown in an adult *trp* photoreceptor with 0.5 mM bath Ca^{2+} (Cs/TEA Cl electrode). (*B*) Current-voltage plot of the rundown current from traces in *A* determined from the instantaneous currents (1 ms after the voltage step; ■) and the steady-state (average current between 25 and 50 ms after voltage step; □). As in WT (c.f. Fig. 2 *D*) there is still a marked outward rectification, but this is now characterized by significant noise levels, and develops more rapidly. Hyperpolarizing steps induce small rapid transient inward currents, but there is little inward rectification.

As in WT photoreceptors, rundown currents in *trp* appear to be carried by cations: the reversal potential (determined by subtracting i-V curves before and after rundown) is close to zero (−0.8 ± 2.3 mV [n = 15] in 0.5 mM Ca^{2+} Ringer) which is similar to the E_{rev} of the light response in *trp* flies before rundown (Hardie and Minke, 1992). The RDC also appears essentially the same whether recorded with Cs/TEA Cl, KCl, or K-gluconate electrodes.

Residual Light Responses after Rundown

As seen already in Fig. 1 *C*, responses to light can usually still be elicited after rundown. However, these show a number of significant differences from normal responses recorded before rundown (Fig. 4): (*a*) sensitivity is reduced by at least 100-fold; (*b*) the responses at negative holding potentials have much slower kinetics; (*c*) responses no longer show the typical voltage dependent kinetics of the normal response (Hardie, 1991*a*); (*d*) the characteristic inward rectification of the normal

light response (Hardie, 1991*a*) is absent; (*e*) as already mentioned, the E_{rev} is shifted by ~10–15 mV to more negative values (E_{rev} with 2 mM external Ca^{2+}: before rundown 13.8 ± 1.4 mV; immediately after rundown 2.4 ± 1.2 mV [n = 7]). In *trp* flies, no response to light could be detected after rundown.

Noise Analysis of the RDC

If the RDC reflects spontaneous channel activity, the noise power spectra should be fitted by simple Lorentzian functions. Analysis of the noise can then be used to

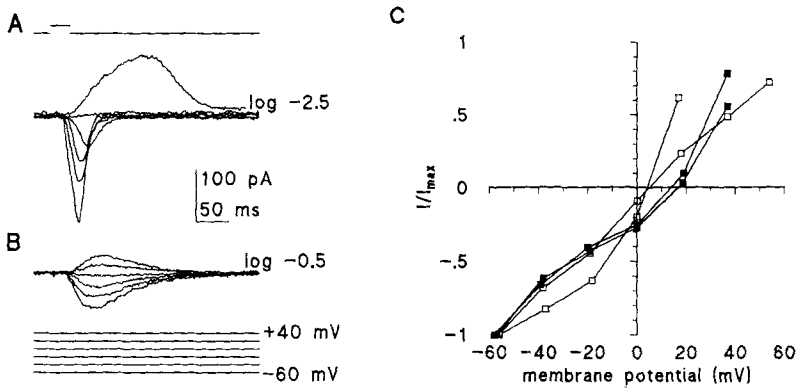

FIGURE 4. Responses to flashes of light recorded at different holding potentials (from −60 mV to +40 mV in 20-mV steps) before and after rundown in adult WT photoreceptor. Bath Ringer 2 mM Ca^{2+}. (*A*) Before rundown response kinetics show a marked voltage dependence and reverse at ~ +15 mV (Hardie, 1991*a*). (*B*) After rundown, responses are at least 2 log units less sensitive, kinetics are slower and voltage independent; reversal potential is now shifted to near 0 mV. Top trace is stimulus marker. (*C*) Current-voltage relationship determined from peak responses in *A* and *B* plus one other cell before (*solid symbols*) and after (*open symbols*) rundown. Responses normalized to response at −60 mV and corrected for series resistance errors. Note that, apart from the shift in reversal potential, the inward rectification is reduced or absent after rundown. Outward rectification is still apparent in one cell, but often could not be reliably demonstrated as sensitivity continues to decline following rundown (i-V series performed starting at −60 mV).

extract information about the underlying channel open times and conductances, although bearing in mind the uncertainty of the underlying assumptions, the accuracy of the derived parameters should be treated with some caution.

WT. For noise analysis, recordings were made at −60 mV, at a variety of external Ca^{2+} concentrations. To improve temporal resolution, measurements were often made in *ninaE* mutants where the reduced membrane surface area results in a faster clamp time constant than in WT (see Materials and Methods). As for other characteristics of the RDC, the noise properties appeared the same whether recorded with pipettes containing Cs/TEA Cl, KCl, or K gluconate. Power spectra were obtained by Fourier transforming noise records sampled during the steady state of

the RDC (Fig. 5; see Materials and Methods). In a few cases the spectra were well fitted by a single Lorentzian function:

$$\text{Power spectral density} = 1/[1 + (2\pi \cdot f \cdot \tau)^2] \quad (2)$$

where f = frequency, and time constant, τ, was in the range 1–2 ms. This would be consistent with a single class of channel with mean open time, τ. In most cases, however, the power spectra also required the linear addition of an appropriately weighted second Lorentzian component, i.e.:

$$\text{Power spectral density} = w_1\{1/[1 + (2\pi \cdot f \cdot \tau_1)^2]\} + w_2\{1/[1 + (2\pi \cdot f \cdot \tau_2)^2]\} \quad (3)$$

Where w_1 and w_2 are weighting factors. τ_1, always the dominant component, was again in the range 1–2 ms, while τ_2 lay between 0.2 and 0.5 ms. This might indicate a second class of channel or a different open state of the same channel. The slower time constant (τ_1) at least, showed little difference for a range of external Ca^{2+} concentrations between 0 and 2 mM (see Fig. 7). Significantly, and in marked contrast to the power spectrum of light-induced noise (Fig. 5 *C;* Wong & Knight, 1980; Barash, Suss, Stavenga, Rubinstein, and Minke, 1988), the roll-off of the rundown noise power spectra was never seen to be steeper than a single Lorentzian. This is thus consistent with the suggestion that the RDC reflects the random openings of channels uncoupled from the amplification stages of the transduction cascade.

For noise generated by random channel openings, the ratio between the variance in the noise and the mean amplitude of the current should be a measure of the underlying single channel currents (Colqhoun and Hawkes, 1977). If more than one class of channel is activated then the derived value will reflect the appropriately weighted contributions of the different channel classes. This analysis assumes that channel open probability is small, in which case the variance/mean ratio should be constant for different values of the mean. In order to obtain data at different mean amplitudes, noise was sampled and analyzed at different times during the development of the rundown current. The resulting plots of variance against the mean (Fig. 6) show that the variance/mean ratio is indeed relatively constant. An exception was sometimes found during the first second of rundown in WT (or *ninaE*) photoreceptors when the variance could be up to two or three times larger than expected. This might suggest that initially the noise is amplified by step(s) of the phototransduction cascade or that different classes of channels (see below) are activated with different time courses. With this exception variance/mean ratios in WT (or *ninaE*) photoreceptors are rather low and indicate an apparent single-channel conductance of 1.5–4.5 pS with 0.5 or 2 mM external Ca^{2+} (mean 2.9 ± 1.1 pS [n = 11] at 0.5 mM Ca^{2+}). As the external Ca^{2+} concentration is reduced the apparent conductance became even lower (see Fig. 7), and with nominally Ca^{2+} free Ringer was only 0.7 ± 0.3 pS (n = 3).

trp. As is readily seen with the naked eye (Figs. 1 *B* and 6 *A*), rundown currents in *trp* are characterized by more prominent high frequency noise fluctuations than in WT. This is reflected in the variance/mean ratios which suggest an average single channel event between 12 and 30 pS (mean = 25.8 ± 9.5 pS [n = 8] with 0.5 mM

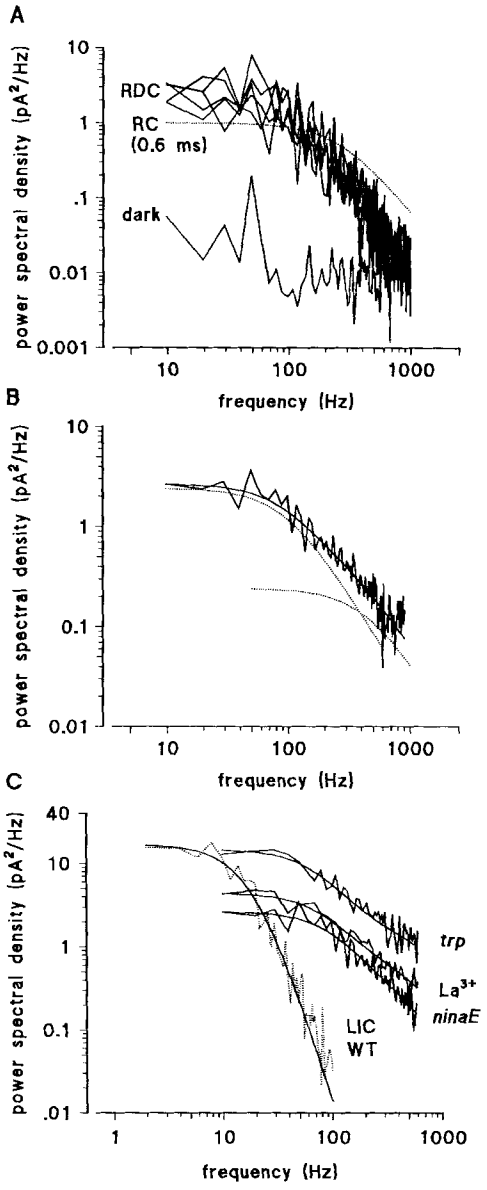

FIGURE 5. Power spectra determined from rundown currents. (*A*) Raw, Fourier-transformed power spectra in *ninaE* p15 photoreceptor: before rundown (dark) and during the steady state after rundown (five spectra superimposed). The dotted curve (RC) is the filtering function of the clamp time constant (0.60 ms) in this cell. (*B*) Averaged and corrected power spectrum from data in *A:* a total of 11 raw spectra have been averaged and the dark noise spectrum subtracted. Finally, the data have been corrected for the clamp time constant by dividing by the RC filter function (Eq. 1). The data have been fitted (*solid curve*) by the weighted sum of two Lorentzian functions (Eq. 3) with time constants, τ_1 1.65 ms (w_1 = 91%) and τ_2 = 0.35 ms (w_2 = 9%). *dotted lines:* single Lorentzian components of the fit. C: the same ninaE power spectra compared with corrected RDC power spectra from adult photoreceptors in *trp* and also in WT recorded in the presence of 20 μM La^{3+}. Both have been fitted by the sums of two Lorentzians with time constants in a similar range (*trp* 2.35 and 0.19 ms, and La^{3+} 1.98 and 0.26 ms). The power spectrum of light-induced noise from a WT photoreceptor is also shown (sampled and filtered at lower frequencies). Power is concentrated at much lower frequencies, the roll-off is considerably steeper than a single Lorentzian and has been fitted by a power of Lorentzian function:

$$\text{power spectral density} = 1/[1 + (2\pi \cdot f \cdot \tau)^2]^n$$

with n = 2.1, and τ = 8.3 ms). All data recorded in 0.5 mM Ca^{2+} Ringer, holding potential of −60 mV. Pipette solution KCl (K-gluconate for *ninaE* fly).

Ca^{2+}; Fig. 7). By contrast, power spectra from the rundown noise in *trp* were very similar to those recorded in WT (Fig. 5 *C;* see also Table I), with no significant differences in the derived mean open times, or the tendency to require a second Lorentzian component to fit the data. As discussed below, this raises the possibility that the rundown noise in WT is dominated by the non–*trp*-dependent channels and that the *trp*-dependent channels are essentially noise-free.

Although La^{3+} applied *after* rundown has developed in WT almost completely blocks the RDC (Fig. 2), RDCs do sometimes develop in WT flies in the presence of La^{3+} (10–20 μM). The behavior appeared indistinguishable from that in *trp:* i.e., rundown currents either did not develop at all or were rather small (192 ± 95 pA [n = 3]), variance/mean ratios indicated single channel conductances of ~20–30 pS

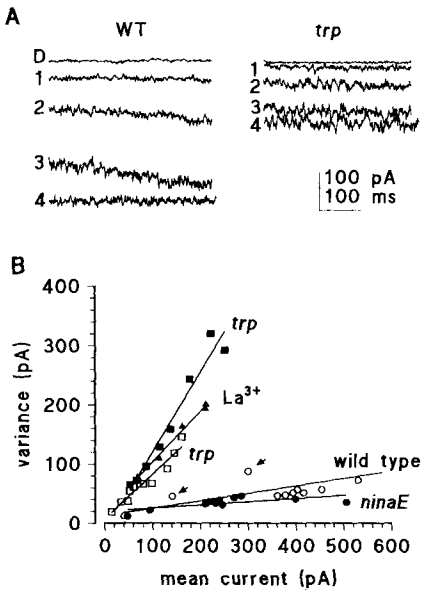

FIGURE 6. Variance in the RDC noise. (*A*) Representative samples of noise in the dark before rundown (*D*) and during the development of the rundown current in WT (left; traces *1–3* during rising phase, *4* in steady state); and *trp* photoreceptors (right, *1–4* all during rising phase). In both cases there is an associated high frequency noise, but the relative noise is clearly greater in *trp*. All data recorded in 0.5 mM Ca^{2+} Ringer, WT with Cs TEA Cl electrode, *trp* with KCl. (*B*) Variance of the noise signal plotted against the mean current in WT (○) *ninaE* (●), WT in the presence of 20 μM La^{3+} (▲), and *trp* (two cells, ■, □). The plots are approximately linear and have been fitted by regression lines the slopes (variance/mean) of which can be taken as a measure of the amplitude of the underlying events. In the WT photoreceptor, the variance/mean ratios measured during the first 1 or 2 s of rundown (arrows correspond to traces 2 and 3 in *A*) were higher than at later times.

(28.7 ± 5.7 pS [n = 3]), and power spectra were indistinguishable from WT or *trp* flies (Table I and Figs. 5 and 6).

DISCUSSION

Our recent study of the light-induced current in WT and *trp* photoreceptors suggested that at least two functional classes of channels with different ionic selectivities underlie the light response in *Drosophila* photoreceptors. One of these appears to be encoded, at least in part, by the *trp* gene and is blocked by La^{3+} (Hardie and Minke, 1992; Phillips et al., 1992). In principle, however, it could be postulated that there is a single channel class that undergoes dynamic modulation in

ionic selectivity. Such a hypothesis, however, would need to assume that channels constructed without the *trp* gene product (whose primary structure suggests it is a single subunit of a tetrameric channel; Phillips et al., 1992) have a lower Ca^{2+} permeability which cannot be modulated. Furthermore, it would need to be assumed that these consequences of an altered channel subunit composition are quantitatively mimicked in the WT channel by extracellular application of La^{3+}. The present data, which show significant differences between the characteristics of spontaneous channel activity of WT and *trp* photoreceptors add further support to the suggestion that there are indeed at least two functionally distinct light-sensitive channels in *Drosophila*. The following discussion will assume that this most direct interpretation of the data is the case.

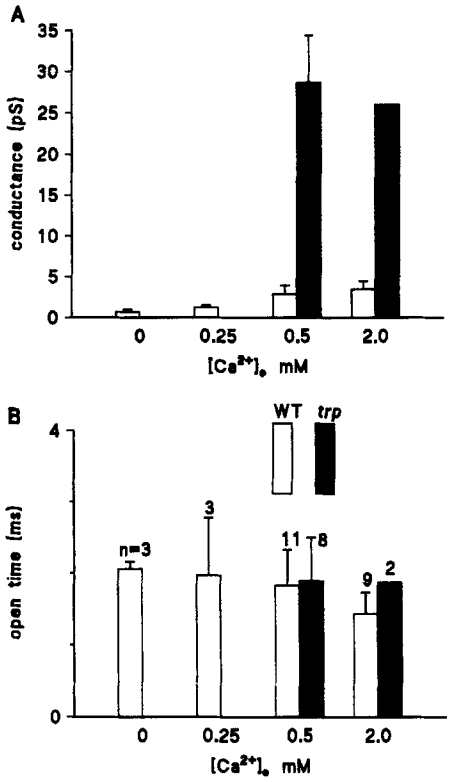

FIGURE 7. Bar graphs showing estimates of (*A*) effective single channel conductance and (*B*) channel open times in WT and *trp* at different Ca^{2+} concentrations. All values derived from analysis of rundown noise (see Figs. 6 and 7). Notice that channel open time in *trp* and WT are indistinguishable and show little if any dependence on external Ca^{2+}. Estimated single channel conductances in *trp* are ~10× greater than in WT. The apparent channel conductance in WT is significantly reduced when external Ca^{2+} is lowered (value at 2 mM Ca^{2+} = 3.5 pS; in 0 Ca 0.7 pS). Data presented as means ± SD, number of cells, *n* indicated on top of bars. In most cases power spectra required two Lorentzians (see Figs. 6 and 7, Table I); only the slower of the two time constants (τ_1, Eq. 3) is plotted.

The RDC shows a similar absolute magnitude, reversal potential, and voltage dependence to the LIC in WT. In addition it is profoundly affected by La^{3+} and the *trp* mutation, both of which affect one class of light-sensitive channel. This leads us to conclude that the RDC is carried by the same channels responsible, at least in part, for the LIC. The power spectra of the steady-state noise in the RDC are dominated by high-frequency components which can be adequately fitted by a single Lorentzian or the weighted sum of two Lorentzians. This is in marked contrast to the LIC, where

the power density is dominated by lower frequencies. Furthermore, light-induced noise spectra show a steeper roll-off that can only be fitted by a Lorentzian raised to a power (Wong and Knight, 1980; Fig. 5), a feature which is attributed to the amplification and consequent filtering properties of the transduction cascade (Wong and Knight, 1980). The noise properties of the RDC are thus consistent with random channel openings effectively uncoupled from the transduction cascade.

Even after rundown a residual light response can be elicited in WT photoreceptors, although not in *trp*. Presently we cannot decide whether this is carried by *trp* or non-*trp*-dependent channels. The properties of this residual light response appear quite distinct from the normal WT light response, in particular having a negatively shifted reversal potential which might suggest involvement of the non-*trp*-dependent channels (Hardie and Minke, 1992). However, the RDC itself (which appears to be largely dominated by *trp*-dependent channels; see below) also undergoes a similar shift in reversal potential, and an alternative explanation may be a redistribution of

TABLE I

Parameters Extracted from Noise Analysis of the RDC in WT or ninaE Photoreceptors (WT), trp, and WT Treated with 10 or 20 μM La^{3+}

	Effective channel conductance	Channel-open times		w_1/w_2
		τ_1	τ_2	
	pS	*ms*	*ms*	
WT (n = 11)	2.9 ± 1.1	1.83 ± 0.50	0.24 ± 0.15	4.6 ± 3.7
trp (n = 8)	25.8 ± 9.5	1.86 ± 0.57	0.23 ± 0.09	6.6 ± 2.4
WT plus La^{3+} (n = 3)	28.7 ± 5.7	1.57 ± 0.33	0.24	7.8

All data from RDCs were recorded at −60 mV in 0.5 mM Ca^{2+} Ringer. Apparent channel conductances were calculated as chord conductances from variance/mean ratio and corrected for filtering effect of clamp time constant (see Materials and Methods). Most of the power spectra were fitted by the sum of two Lorentzian functions (Eq. 3; see Fig. 5): τ_1 and τ_2 are their time constants ("channel open times"); w_1/w_2 their relative weighting. The power spectra of two *trp*, one WT and one of the La^{3+}-treated WT photoreceptors were adequately fitted by a single Lorentzian function (Eq. 2). Apparent single-channel conductances estimated in *trp* and La^{3+}-treated photoreceptors are ~10-fold greater than in WT; however, the frequency characteristics of the power spectra are indistinguishable.

ions owing to the large currents flowing during rundown. The lack of voltage dependent response kinetics is also reminiscent of the *trp* phenotype (Hardie and Minke, 1992); however, this may also be misleading. The voltage dependent kinetics of the normal light response are probably mediated by sequential positive and negative feedback at a late stage in transduction (Hardie, 1991*a*; Hardie and Minke, 1994). A prime candidate is the $InsP_3$ receptor (Ca^{2+} release channel) which in other systems shows a bell-shaped dependency on cytosolic Ca^{2+} in the submicromolar range (Baumann and Walz, 1989; Bezprozvanny, Watras, and Ehrlich, 1991; Finch, Turner, and Goldin, 1991). Presumably if Ca^{2+} rises outside this range, such feedback would be circumvented.

Interpretation of Noise in the RDC

It seems likely that the RDC in the *trp* mutant represents spontaneous activation of the non-*trp*-dependent light-sensitive channels remaining in the mutant. In particu-

lar, the reversal potential of the RDC is indistinguishable from that of the LIC in *trp*, and both RDC and LIC in *trp* show a marked outward rectification with little inward rectification. Analysis of noise variance suggests that these non-selective cation channels have an apparent unitary conductance as large as ~30 pS. An estimate for the mean open time of ~1–2 ms (plus a minor component at higher frequencies) is obtained from the Lorentzian fits to power spectra calculated from the steady-state noise. Some variability was observed in the apparent single–channel conductance (10–30 pS); possibly this reflects the filtering effect of the clamp time constant. Although this was corrected for (see Materials and Methods), the calculated correction factor is particularly sensitive to the estimated contribution of the second, higher frequency component of the power spectra (τ_2 in Eq. 3), which could not be determined with any great confidence.

The RDC in WT photoreceptors is almost completely blocked by La^{3+}. Although this suggests that the RDC is largely mediated by the *trp*-dependent channels, as discussed below, there are reasons for suggesting that the non–*trp*-dependent channels may be gated, directly or via an intermediate, by Ca^{2+}. An alternative suggestion therefore would be that both channels are activated during rundown, but that the non–*trp* channels require the influx of Ca^{2+} via the *trp*-dependent channels to be activated, in which case they would also be inactivated by the La^{3+} blockade. In fact, the similarity of the power spectra of *trp* and WT RDCs suggests that the *noise* in the WT RDC may actually be dominated by the contribution of identical channels to those generating the RDC in the *trp* mutant, whereas essentially noise-free (low conductance) channels carry the majority of the current. This would also be consistent with the observation that the apparent single conductance decreases as extracellular Ca^{2+} is lowered (Fig. 7), reflecting a decreasing contribution of the higher conductance non–*trp*-dependent channels. Note that since the effective channel conductance estimated in WT is ~10% of that in *trp*, if this (our favored explanation) is valid, it still suggests that at least 90% of the WT RDC is carried by *trp*-dependent channels. Since the absolute variance in RDC noise in *trp* photoreceptors is typically greater than in WT (e.g., Figs. 1 and 6), this explanation also implies that a smaller number of non–*trp*-dependent channels are activated during rundown in WT than in the *trp* mutant.

Analysis of noise variance in the RDC in WT yields a much smaller apparent single-channel conductance (≈3 pS) than in *trp*. If, as suggested above, this noise actually represents the contribution of non–*trp* channels activated by Ca^{2+} influx, then the *trp*-dependent channels would presumably be of even lower unit conductance—at most 0.7 pS, the value estimated with Ca^{2+}-free Ringer. Single light-dependent channels previously recorded in invertebrate photoreceptors range in size from ~6 to 40 pS (reviewed in Nagy, 1991); however, channels of the size suggested here would obviously be very hard to detect in single-channel recordings. Furthermore, in *Limulus* the light-sensitive channels are reported, at most, to be only weakly permeable to Ca^{2+} (Deckert and Stieve, 1991) and hence may belong to a distinct class from the *trp*-dependent channels of *Drosophila*. An interesting functional precedent for channels of such low conductance is provided by the cyclic GMP-gated channel responsible for the light response in vertebrate rods (reviewed in Yau and Baylor, 1989). Here it has been argued that the low conductance (in the presence of

external divalent cations) is an adaptation to improve signal-to-noise ratio, by allowing a large number of channels to be active during the light response (Attwell, 1986). Possibly more directly relevant examples include a Ca^{2+} and $InsP_4$ activated Ca^{2+} channel (~2.5 pS) believed to be responsible for phosphoinositide-mediated Ca^{2+} entry in bovine endothelial cells (Lückhoff and Clapham, 1992), and a Ca^{2+} conductance believed to be activated by Ca^{2+} release in mast cells (calcium release-activated calcium conductance or *CRAC*), where channel-associated noise variance was undetectable in whole-cell recordings (Hoth and Penner, 1993).

In principle, an alternative explanation for the apparent small conductance of the *trp*-dependent channels could be that the channel open times fall outside the frequency bandwidths used in our noise analysis (< 10 Hz or > 1000 Hz). Although we cannot exclude a larger conductance channel with open time in the microsecond range, it seems unlikely that very long open times could be involved as a slow noise component would almost certainly have been detected by eye and would be difficult to reconcile with the temporal resolution of phototransduction. It might also be argued that the Ca^{2+} influx during the RDC could elicit a significant electrogenic Na^+/Ca^{2+} exchange current which would then represent an essentially noise-free component to the RDC resulting in underestimation of the single-channel conductance. However, such a large Na^+/Ca^{2+} exchange current would be unrealistic, and under conditions expected to block any possible contribution of Na^+/Ca^{2+} exchange, namely replacing extracellular Na^+ with Li^+ and omitting Ca^{2+} (to prevent reverse Na/Ca^{2+} exchange), RDCs (in WT) were as large, and the variance/mean ratios (< 1 pS) similar or *smaller* than in controls (data not shown).

Mechanism of Channel Activation

In *trp* the light response decays to baseline during continuous bright illumination (Cosens and Manning, 1969; Minke, Wu, and Pak, 1975). Since the primary defect in the *trp* mutant is the absence of a plasma membrane light-dependent Ca^{2+} conductance (Hardie and Minke, 1992), we have previously suggested that the decay in *trp* represents the depletion of intracellular $InsP_3$-sensitive Ca^{2+} stores and that these normally require Ca^{2+} influx via the *trp*-dependent channels to be refilled (Minke and Selinger, 1991; Hardie and Minke, 1992). This interpretation implies that the non-*trp* channels may be activated (possibly directly gated) by Ca^{2+}. This would be consistent with the suggestion that the noise in the WT RDC reflects the contribution of the non-*trp* channels activated by Ca^{2+} influx and explain why the noise variance is reduced when extracellular Ca^{2+} is reduced to nominally zero levels (when we expect that the RDC is almost entirely dominated by the *trp*-dependent channels). On this view, the non-*trp* channels might represent Ca^{2+}-activated nonselective cation channels (CAN channels; reviewed in Partridge and Swandulla, 1988).

By contrast the *trp*-dependent channels appear to remain highly activated in the absence of both internal and external Ca^{2+} and in fact appear to be subject to Ca^{2+}-mediated inactivation (Hardie and Minke, 1994). The mechanism of their activation remains speculative and a detailed discussion, beyond the scope of the present study. We recall however, that the *trp*-dependent channels are highly permeable to Ca^{2+} (Hardie and Minke, 1992); since they are believed to be activated by the phosphoinositide cascade, we recently suggested that their mechanism of

activation may be similar to that responsible for the phenomenon of phosphoinositide-mediated Ca^{2+} entry in a variety of vertebrate cells (Hardie and Minke, 1992). Like invertebrate phototransduction, the mechanism underlying this process is unresolved. However, current models which attempt to explain this process, including the "capacitative Ca^{2+} entry" model (reviewed in Putney, 1990, 1992), and the suggestion that Ca^{2+} entry is triggered by a protein-protein interaction between the $InsP_3$ receptor and the plasma membrane Ca^{2+} channel (reviewed in Irvine, 1991, 1992), raise possible mechanisms of excitation in invertebrate photoreceptors which had not previously been explicitly discussed.

Finally, we note that an important experimental approach for future progress in the analysis of invertebrate phototransduction is likely to involve a search for agents that directly activate the light-dependent channels. The partial characterization of channel properties provided in this and the companion study (Hardie and Minke, 1993) should provide a valuable database for evaluation of the effects of agents or procedures which lead to activation of the different classes of light-dependent channels.

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