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The localization of calcium release by inositol trisphosphate in *Limulus* photoreceptors and its control by negative feedback

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[Plate 1]

Microvillar photoreceptors of invertebrates exhibit a light-induced rise in the intracellular concentration of free calcium (Ca_i) that results in part from release of calcium from an intracellular compartment. This light-induced release of calcium appears to result from a cascade of reactions that involve rhodopsin, a GTP-binding protein and a phospholipase-C which releases inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) from the plasma membrane; the Ins(1,4,5)P_3 acts to release calcium from smooth endoplasmic reticulum. In the ventral photoreceptor of the horseshoe crab *Limulus polyphemus* not all of the endoplasmic reticulum is subject to calcium release by Ins(1,4,5)P_3. Only endoplasmic reticulum in the light-sensitive region of the cell is competent to release calcium in response to Ins(1,4,5)P_3. The release of calcium by Ins(1,4,5)P_3 in ventral photoreceptors appears to be subject to feedback inhibition through elevated Ca_i. We suggest that this feedback inhibition contributes to sensory adaptation in the photoreceptor and may account for oscillatory membrane responses sometimes observed with large injections of Ins(1,4,5)P_3.

1. Introduction

A flash of light delivered to a dark-adapted photoreceptor of an invertebrate causes a rapid rise in the intracellular concentration of free calcium ions, Ca_i (Brown & Blinks 1974; see review by Payne 1986). This rise in Ca_i is thought to be a signal that mediates adaptation and also possibly contributes to the excitation of the photoreceptor by light. In the ventral photoreceptors of the horseshoe crab *Limulus polyphemus*, much of the rise in Ca_i is a result of the release of calcium from internal stores (Brown & Blinks 1974; Levy & Fein 1985). Recent evidence, to be summarized in this paper, suggests that the release of calcium is triggered by the light-induced production of inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3). The production of Ins(1,4,5)P_3 is catalysed by a phospholipase C that is thought to be activated by a G-protein. The catalysis by photoactivated rhodopsin of the exchange of GTP for GDP bound to the G-protein activates the G-protein and initiates the cascade of reactions that release calcium. Thus the biochemical mechanism mediating light-induced release of calcium in the photoreceptors of invertebrates is similar to that mediating the release of calcium in hormonally activated cells

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(see reviews by Berridge 1987; Cockcroft 1987). In the first part of this paper we will review recent biochemical evidence for the linkage of the mobilization of intracellular calcium to the activation of a G-protein by photoactivated rhodopsin and the consequent production of Ins(1,4,5)P_3.

Two aspects of the actions of Ins(1,4,5)P_3 inside living *Limulus* ventral photoreceptors will be reviewed in the second part of the paper. The first concerns the localization of the actions of Ins(1,4,5)P_3 inside the photoreceptor. The endoplasmic reticulum (ER) localized beneath the light-sensitive microvillar region of the photoreceptor's plasma membrane appears to be sensitive to Ins(1,4,5)P_3, while ER located in other areas of the photoreceptor appears to be less sensitive. The second aspect concerns evidence that the calcium released by Ins(1,4,5)P_3 in ventral photoreceptors can prevent further release of calcium. We suggest that this negative-feedback pathway limits the release of calcium after a small injection of Ins(1,4,5)P_3 to a brief transient. Following the injection of larger quantities of Ins(1,4,5)P_3, the same negative-feedback pathway would be responsible for the sustained oscillations of calcium release that are often recorded. More importantly, this negative-feedback pathway may explain the adaptation of calcium release during sustained illumination. Adaptation of the release of an internal messenger such as Ca_i is a vital component of any sensory transduction pathway.

2. Evidence linking photoactivated rhodopsin to the production of inositol 1,4,5-trisphosphate

Light activates a GTP-binding protein

Rhodopsin, the visual pigment of invertebrate photoreceptors, is concentrated in cylindrical foldings of the plasmalemma, termed microvilli. Microvilli are 50–80 nm in diameter and 1–2 μm in length. A typical photoreceptor bears about 10^5 microvilli (Fein & Szuts 1984), concentrated in one lobe or region of the cell so as to create a large surface area for the efficient absorption of light (figure 1).

Rhodopsin consists of a chromophore (11-*cis* retinal or an analogue) that is covalently bound

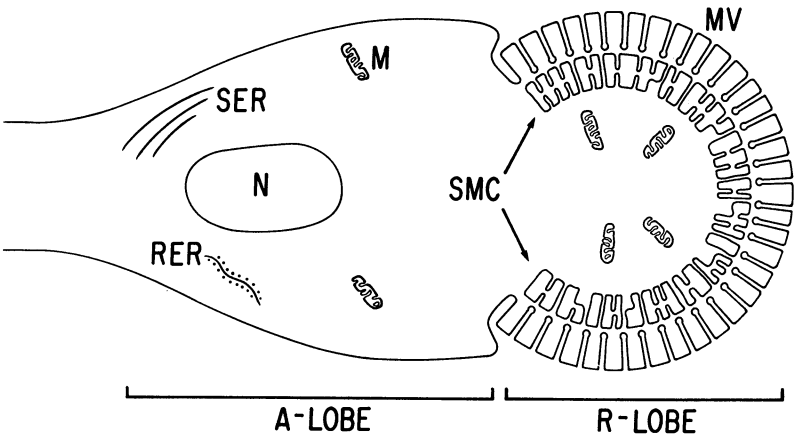

Figure 1. Diagram of a cross section through a *Limulus* ventral photoreceptor, showing the structures in the A- and R-lobes of the photoreceptor, as described by Calman & Chamberlain (1982). The A-lobe contains the nucleus (N), smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER) and mitochondria (M). The R-lobe contains microvilli (MV), submicrovillar cisternae of smooth ER (SMC) and mitochondria.

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to the apoprotein opsin (molecular mass 37–46 kDa; see review by Kirschfeld (1986)). Light initiates the visual process by the photoisomerization of the chromophore from an 11-*cis* to all-*trans* configuration. When photoisomerization of the chromophore occurs, a stable product called metarhodopsin is formed. Metarhodopsin is formed within 1 ms of the absorption of a photon by rhodopsin (Kruizinga *et al.* 1983). During the formation of metarhodopsin a conformational change in the opsin moiety, caused by the alteration in chromophore structure, is presumably transmitted from the hydrophobic core of opsin to the cytoplasmic surface, enabling the interaction of metarhodopsin with a G-protein (see below) and a kinase that phosphorylates metarhodopsin (Paulsen & Hoppe 1978; Paulsen & Bentrop 1984; Vandenberg & Montal 1984*b*). So far these are the only known biochemical reactions in which metarhodopsin participates.

After a receptor catalyses the binding of GTP to a G-protein and so activates it, hydrolysis of the bound GTP returns the G-protein to the inactive state, in which the G-protein has GDP bound to it. Thus a light-activated GTPase activity would be expected in microvillar photoreceptors if photoactivated rhodopsin does indeed activate a G-protein. Such a light-activated GTPase has been reported in membrane preparations of octopus, squid, housefly (*Musca*) and blowfly (*Calliphora*) photoreceptors (Calhoon *et al.* 1980; Vandenberg & Montal 1984*a*; Blumenfeld *et al.* 1985; Paulsen & Bentrop 1986). In all preparations of microvillar membranes, GTPase activity is maintained in the dark for tens of minutes after the initial flash of light, implying that some factor that normally turns off the transduction process is missing. In fly photoreceptors, this prolonged GTPase activity appears to be caused by a metarhodopsin species that remains active long after a flash (Blumenfeld *et al.* 1985; Paulsen & Bentrop 1986). The missing factor may be a soluble protein kinase that normally phosphorylates metarhodopsin. Phosphorylation of metarhodopsin in the rod photoreceptors of vertebrates quenches the reaction between rhodopsin and G-protein (see review by Kühn 1986). A similar phosphorylation of metarhodopsin occurs in the photoreceptors of invertebrates (Paulsen & Hoppe 1978; Vandenberg & Montal 1984*b*; Bentrop & Paulsen 1986) and it has been suggested that phosphorylated metarhodopsin is unable to initiate phototransduction (Paulsen & Bentrop 1984; Lisman 1985; Minke 1986).

Light should activate the G-protein by promoting the binding to the G-protein of GTP in exchange for GDP. Light should also promote the binding to the G-protein of the hydrolysis-resistant analogues of GTP, GTPγS and GMP-PNP. Binding of GTPγS and GMP-PNP has been observed in preparations of squid (Vandenberg & Montal 1984*a*; Robinson & Cote, personal communication) and housefly photoreceptors (Devary *et al.* 1987). Binding saturates at a ratio of rhodopsin to bound GTPγS of between 10:1 and 100:1, indicating a minimum of one G-protein per 10–100 rhodopsin molecules and therefore a minimum of 10–100 G-proteins in a microvillus containing 1000 rhodopsin molecules.

The identification of G-proteins in microvillar membranes has been possible through the use of toxin-catalysed labelling of putative G-proteins. The α subunits of some GTP-binding proteins become ADP-ribosylated in the presence of cholera or pertussis toxins, enabling identification of the G-protein after incubation with the toxin and radiolabelled NAD. Modulation by light of the extent of toxin-catalysed labelling would imply the interaction of the labelled G-protein with rhodopsin. Studies performed on preparations of fly and cephalopod eyes that are enriched in microvillar membrane show at least one polypeptide (molecular mass 41–44 kDa) that is ADP-ribosylated in the presence of one of the two toxins

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in a light-modulated manner (Vandenberg & Montal 1984*a*; Bentrop & Paulsen 1986; Tsuda *et al.* 1986). Quantification of cholera-toxin catalysed labelling of the 44 kDa protein in squid photoreceptor membranes (Vandenberg & Montal 1984*a*) predicts a minimum of one G-protein per 45 rhodopsin molecules, in good agreement with the density of G-proteins estimated from GTPγS binding (see above).

As an alternative to toxin-catalysed labelling, G-proteins can be identified by using a radioactively labelled photo-affinity analogue of GTP, azidoanilido-GTP. This technique has recently been applied to crude membrane preparations of housefly photoreceptors (Devary *et al.* 1987). Blue light, which induces a net conversion of rhodopsin to metarhodopsin, promotes the binding of azidoanilido-GTP to a polypeptide of molecular mass 41 kDa, which may be homologous with the 41 kDa light-dependent cholera-toxin substrate associated with purified blowfly microvillar membranes.

Light activates a phospholipase C and produces Ins(1,4,5)P_3

Figure 2 illustrates the scheme proposed by Fein (1986), which links photoactived rhodopsin via a G-protein to the production of Ins(1,4,5)P_3. Light-induced production of InsP_3 has been reported in whole *Limulus* ventral eye (Brown *et al.* 1984), whole squid retina (Szuts *et al.* 1986; Brown *et al.* 1987) and in membrane preparations from squid and housefly eye (Wood *et al.* 1987*a*; Devary *et al.* 1987). These experiments confirm earlier reports of phosphoinositide turnover in cephalopod retinae (Vandenberg & Montal 1984*b*; Yoshioka *et al.* 1984). Production of Ins(1,4,5)P_3 after a flash is rapid; elevations of Ins(1,4,5)P_3 content are observed in squid retinae within 200 ms (Szuts *et al.* 1986). Thus production of

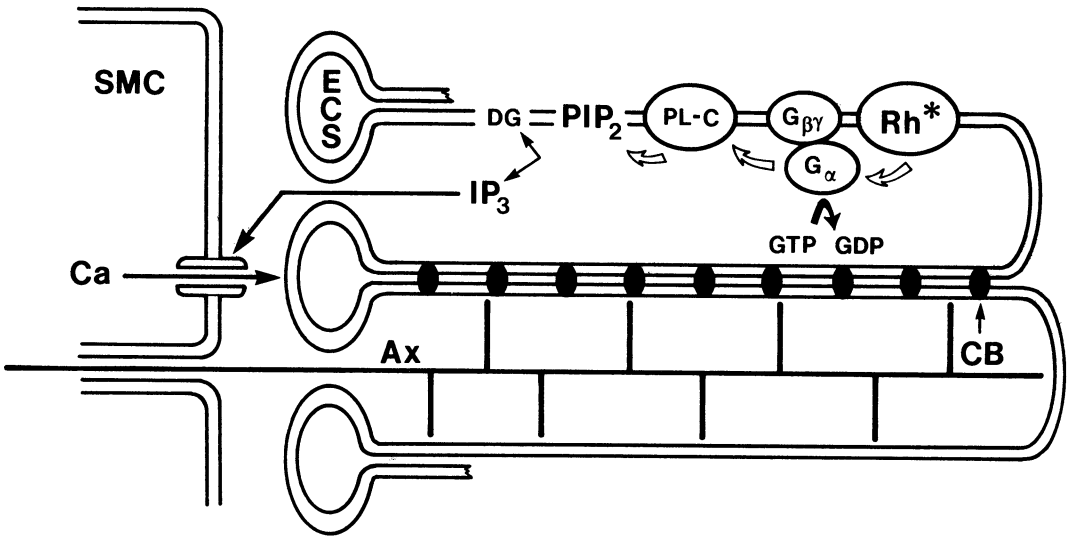

FIGURE 2. Diagram showing two microvilli. The upper microvillus illustrates the scheme proposed by Fein (1986) for linking photo-activated rhodopsin (Rh*) to the production of Ins(1,4,5)P_3 (IP_3). Rh* first catalyses the exchange of GTP for bound GDP on the alpha subunit of a G-protein (G_α). $G_{\beta\gamma}$ represents the β_γ subunits of the G-protein. G_α, with GTP bound, then activates a phospholipase-C (PL-C) which catalyses the hydrolysis of (PtdIns(4,5)P_2) into Ins(1,4,5)P_3 and diacylglycerol (DG). Ins(1,4,5)P_3 then diffuses down the microvillus to cross to the neighbouring cisternae of smooth ER (SMC), from which it releases calcium. The lower microvillus is drawn to illustrate cytoskeletal components. A thin axial filament (Ax), possibly made of actin (Blest *et al.* 1982; Saibil 1982) is linked to the plasma membrane by sidearms. Cross-bridges (CB) join the plasma membranes of apposing microvilli (Saibil & Hewat 1987), leaving very little extracellular space (ECS) between microvilli except at their bases.

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Ins(1,4,5)P_3 in squid eyes occurs over the same timescale as release of calcium in *Limulus* ventral photoreceptors (see below). Light-induced decreases in phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) the lipid precursor of Ins(1,4,5)P_3, have been observed in squid and *Limulus* preparations (Vandenberg & Montal 1984*b*; Brown *et al.* 1984, 1987) suggesting that the Ins(1,4,5)P_3 is produced, as in other cells, by the activation of a phospholipase C which hydrolyses PtdIns(4,5)P_2. This has been directly confirmed in membrane preparations from squid photoreceptors by observing light-activated hydrolysis of exogenous PtdIns(4,5)P_2 (Baer & Saibil 1987).

In most of these preparations, the content of InsP_2 and InsP_1 also rises after a light flash. These inositol phosphates probably arise from sequential hydrolysis of Ins(1,4,5)P_3. In *Limulus* and squid preparations the content of PtdIns(4,5)P_2 falls after illumination whereas that of PtdIns4*P* is not significantly reduced (Brown *et al.* 1984, 1987). This suggests that the initial action of light is the hydrolysis of PtdIns(4,5)P_2 to produce Ins(1,4,5)P_3, which is then degraded to InsP_2 and InsP_1 (Storey *et al.* 1984). This interpretation is supported by the observation that production of InsP_3 in outer segments of squid retinae after a flash precedes that of InsP_2 (S. F. Wood, personal communication). The production of InsP_2 in the latter preparation and in a preparation of housefly photoreceptor membranes (Devary *et al.* 1987) can be inhibited by the addition of 2,3-diphosphoglyceric acid, which inhibits the phosphatase that hydrolyses Ins(1,4,5)P_3 (Downes *et al.* 1982).

Although some of the Ins(1,4,5)P_3 is degraded to the less active Ins(1,4)P_2 it is possible that, in some preparations, some Ins(1,4,5)P_3 is phosphorylated to Ins(1,3,4,5)P_4. Illumination of *Limulus* ventral eyes (Irvine *et al.* 1985) (but not squid eyes (Szuts *et al.* 1986; Brown *et al.* 1987)) results in the production of Ins(1,3,4)P_3 in addition to Ins(1,4,5)P_3. Ins(1,3,4)P_3 is thought to be formed by dephosphorylation of Ins(1,3,4,5)P_4 (Irvine *et al.* 1986). However, InsP_4 has not so far been detected in photoreceptors of invertebrates.

The light-activated G-protein may couple photoactivated rhodopsin to phospholipase C

Several groups of workers are now trying to obtain evidence that rhodopsin is coupled to the activation of phospholipase C via the light-activated GTP-binding protein (Fein 1986). The effects on inositol phosphate production of fluoride, GTPγS and GDPβS have been examined. Fluoride and GTPγS are known to activate GTP-binding proteins such as transducin (Stein *et al.* 1981; Yamanaka *et al.* 1985), and when introduced into invertebrate photoreceptors they mimic the ability of light to activate the photoreceptor's electrical response (Fein & Corson 1979, 1981; Bolsover & Brown 1982; Payne 1982; Minke & Stephenson 1985). GDPβS inhibits the activation of G-protein (Eckstein *et al.* 1979) and inhibits phototransduction in *Limulus* ventral photoreceptors (Fein 1986). In biochemical studies of InsP_3 production, preparations of housefly and squid photoreceptor membranes have so far been examined. Devary *et al.* (1987) report that GTPγS enhanced the accumulation of InsP_2 following a light flash. Rapid hydrolysis of InsP_3 to InsP_2 in this preparation prevented the analysis of InsP_3 content. Fluoride caused a similar accumulation of InsP_2 in darkness, and addition of GDPβS completely inhibited the accumulation of InsP_2 after illumination or treatment with fluoride. Fluoride has also been applied to a preparation of squid photoreceptor membranes (Wood *et al.* 1987*b*). Increased content of InsP_3 and accumulation of InsP_2 were observed after incubation with fluoride in darkness. A correspondingly decreased PtdIns(4,5)P_2 content suggests that, like light, fluoride activates phospholipase C.

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A more direct demonstration of the GTP dependence of InsP_3 production is prevented in preparations of crude photoreceptor membranes by the presence of contaminating micromolar GTP, which is sufficient to sustain the GTPase activity even if no GTP is added. To overcome this problem, Baer & Saibil (1987) have developed a purified preparation of squid photoreceptors that is washed to remove endogenous GTP. Radioactively labelled PtdIns(4,5)P_2 is added as a substrate for the light-activated phospholipase C. At a calcium concentration of 1 μM, light-activated production of InsP_3 showed an absolute requirement for GTP.

The above studies demonstrate that invertebrate photoreceptors possess the biochemical machinery needed for the light-induced production of InsP_3 in accordance with the model of figure 2. We shall now consider the physiological effects of Ins(1,4,5)P_3 within living ventral photoreceptors of *Limulus*, concentrating on two aspects: the localization of the actions of Ins(1,4,5)P_3 within the cell, and the control of calcium release by a negative-feedback pathway.

3. The localization of calcium release within *Limulus* ventral photoreceptors and the actions of calcium during phototransduction

Light-induced calcium release is localized within Limulus ventral photoreceptors

The ventral photoreceptors of *Limulus* are clearly segmented into two lobes (figure 1), only one of which (the rhabdomeral or R-lobe) bears microvilli (figure 3*a*, plate 1) and is therefore light-sensitive (Calman & Chamberlain 1982; Stern *et al.* 1982). The arhabdomeral (A) lobe contains the nucleus but no microvilli and is therefore insensitive to light.

Bright flashes of light rapidly increase Ca_i in *Limulus* ventral photoreceptors (Brown & Blinks 1974; Brown *et al.* 1977; Nagy & Stieve 1983; Levy & Fein 1985). After a brief flash of light, the time course of the rise in Ca_i is very rapid, reaching its peak amplitude of approximately 40 μM in less than 300 ms and declining to half-maximal amplitude within 2 s. The light-induced rise in Ca_i is diminished by less than 50% when *Limulus* ventral photoreceptors are soaked, in darkness, with seawater containing little or no calcium, suggesting that a substantial fraction of the rise in Ca_i is due to the release of calcium from internal stores rather than

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Description of plate 1

Figure 3. (*a*) Low-magnification electron micrograph of a cross section through the rhabdomeral lobe of a *Limulus* ventral photoreceptor. The R-lobe is characterized by distinct external rhabdoms (RH), formed by the photoreceptive microvilli. Submicrovillar cisternae of smooth ER are labelled by arrows. Scale bar, 1 μm.

(*b*) High-magnification electron micrograph of the submicrovillar region in the R-lobe. Note that the submicrovillar cisternae of the smooth ER (ER) are closely juxtaposed to the bases of the microvilli (MV). The ER is separated from the microvillar bases by a gap of less than 100 nm. Narrow cytoplasmic bridges (★) traverse the ER and connect the microvilli to the rest of the cytoplasm. Scale bar 0.1 μm.

(*c*) Cytochemical evidence for the Ca^{2+}-accumulating ability of the SMC of smooth ER. The micrograph is of a cell that was first permeabilized for 15 min by saponin treatment (composition of the lysis medium: KCl 450 mM, $MgCl_2$ 10 mM, ATP. Na_2 5 mM, K_2EGTA 2 mM, imidazole 20 mM, pH 7.0; saponin 500 μg ml^{-1}) and subsequently incubated for 30 min in a loading medium containing *ca.* 8×10^{-7} M free calcium ions, MgATP and oxalate (composition: KCl 425 mM K oxalate 25 mM, $MgCl_2$ 5 mM, ATP.Na_2 5 mM, K_2EGTA 1 mM, CaEGTA 4 mM, imidazole 20 mM, pH 7.0). The cell was prepared for electron microscopy as previously described (Walz 1982*b*). Electron-dense Ca oxalate deposits within the SMC of smooth ER are due to ATP-driven Ca^{2+} uptake and indicate that this organelle is able to accumulate Ca^{2+} actively with high affinity (See Walz 1982*c*). The microvilli (MV) have largely disintegrated as a result of the saponin treatment. Scale bar 1 μm.

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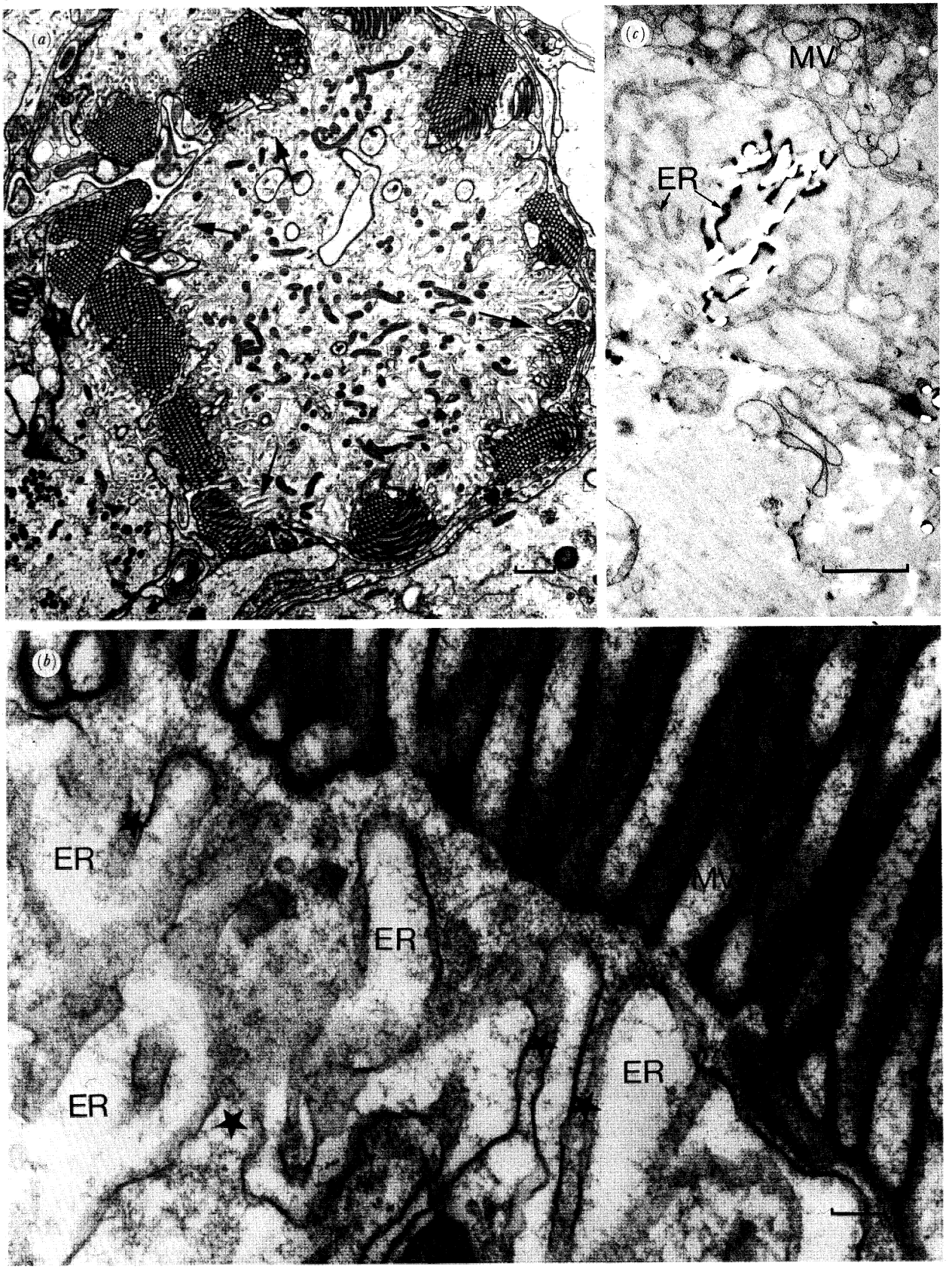

FIGURE 3. For description see opposite.

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entrance of calcium from the bathing medium (Brown & Blinks 1974; Levy & Fein 1985; Bolsover & Brown 1985). The large magnitude of the rise in Ca_i implies that a biochemical amplifier must mediate the release of several thousand calcium ions in response to one photoisomerization (Levy & Fein 1985).

The light-induced rise in calcium is not uniform within the cell (Harary & Brown 1984), but is largely confined to the light-sensitive R-lobe of the cell (Levy & Fein 1985; Payne & Fein 1987*a*). If illumination is confined to a 10–20 μm diameter spot within the 50–100 μm diameter R-lobe, then the rise in Ca_i is further confined to that spot (Fein & Payne 1987*a*). The light-sensitive stores of calcium are therefore localized in the R-lobe and the process that releases those stores is confined to the area of the R-lobe that is illuminated. A second clue as to the site of the light-sensitive calcium store is the speed with which light releases calcium. The approximately 50 ms delay between the absorption of light and the beginning of the rise in Ca_i allows little time for diffusion of a messenger, released from the microvilli, to a distant site within the photoreceptor (Payne & Fein 1988).

A good candidate for the light-sensitive calcium store is the network of smooth ER, the submicrovillar cisternae (SMC), that lie close to the bases of the microvilli (figure 3*b*; see also Clark *et al.* 1969; Lisman & Strong 1979; Calman & Chamberlain 1982; Walz & Fein 1983). These cisternae are ubiquitous features of microvillar photoreceptors and their morphology has been extensively studied (for review see Whittle 1976). The cisternae are ideally localized very close to the bases of the microvilli. The gap between the base of the microvilli and the SMC is less than 100 nm in *Limulus* ventral photoreceptors (figure 3*b*). Narrow cytoplasmic bridges connect the narrow compartment between microvilli and SMC to the rest of the cytoplasm.

Incubation of permeabilized *Limulus* photoreceptors with 800 nM free calcium ions, 5 mM ATP and 25 mM potassium oxalate results in the precipitation of calcium oxalate within the SMC (figure 3*c*). The SMC of *Limulus* ventral photoreceptors, like those of leech and fly (Walz 1979, 1982*a*, *b*, *c*) therefore uses ATP to accumulate calcium actively. Thus the SMC may serve as both a sink and a source for the calcium released by light. This dual role of the SMC may explain the changes in the distribution of Ca_i with time after a flash that can be observed when the light-induced rise in Ca_i is monitored with the luminescent photoprotein aequorin (Shimomura *et al.* 1962). If the aequorin luminescence is monitored with an image intensifier and sequential frames of video recording are photographically summed (Payne & Fein 1987*a*), the average aequorin luminescence is uniform over the R-lobe. However, frame-by-frame analysis of the rise and decay of the luminescence after a flash of light reveals that, in some cells, the luminescence initially appears to grow around the periphery of the R-lobe (figure 4*a*). As luminescence falls, it lingers in the centre (figure 4*d*). This pattern would be expected if calcium were released from the SMC in the periphery of the R-lobe and then rapidly re-accumulated back into the SMC. Future experiments using a more sensitive image-intensifier or the fluorescent calcium indicator fura-2 (Grynkiewicz *et al.* 1984) may reveal a fascinating fine structure to light-induced calcium release and calcium uptake within these cells.

Not all endoplasmic reticulum in Limulus ventral photoreceptors may be sensitive to Ins(1,4,5)P_3

Brief, pulsed, pressure-injections of Ins(1,4,5)P_3 into *Limulus* ventral photoreceptors cause a transient rise in intracellular calcium, similar in amplitude and time course to that produced by a flash of light (figure 5*b*) (see also Brown & Rubin 1984; Payne *et al.* 1986*b*). The rise in Ca_i is not diminished by soaking the photoreceptors in seawater containing EGTA and no

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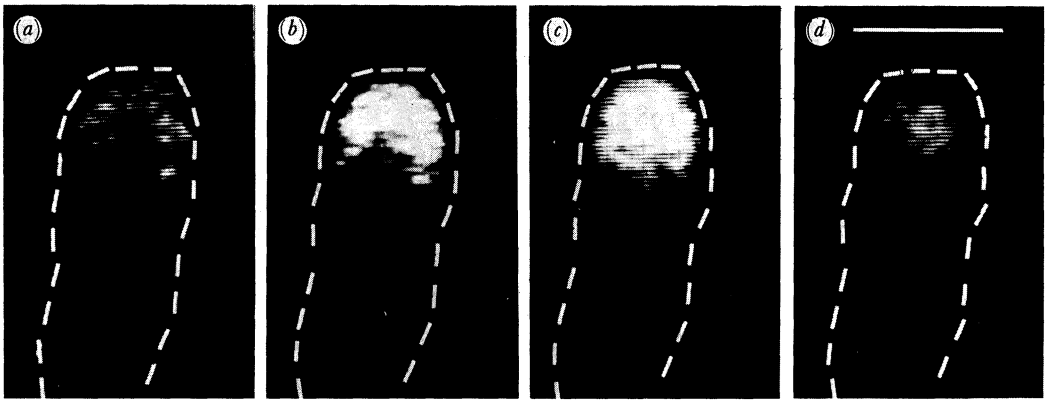

FIGURE 4. Images of aequorin luminescence taken at increasing times after a 10 ms flash was delivered to a *Limulus* ventral photoreceptor. The diffuse flash illuminated the entire field of view. The broken line outlines the cell body, with the axon exiting from the arhabdomeral lobe towards the bottom of each video frame and the rhabdomeral lobe situated towards the top of each frame. Aequorin luminescence is always confined to the distal region of the cell in the R-lobe. Each image is a single video frame, the frames being recorded 230 ms (*a*), 460 ms (*b*), 1160 ms (*c*) and 3000 ms (*d*) after the end of the light flash. Aequorin luminescence begins at the periphery of the R-lobe (*a*), spreads to fill in the centre of the R-lobe (*b–c*) and declines first at the periphery of the R-lobe (*d*). The images were taken with a Nikon M-Plan 20 × N.A. 0.4 microscope objective focused on to the photocathode of a Venus Scientific Instruments TV3M image intensifier. The video output of the intensifier was stored on the video disk of a motion analyser (Sony model SVM-1010) and individual frames were photographed. Scale bar, 50 μm. For further details see Payne & Fein (1987*a*).

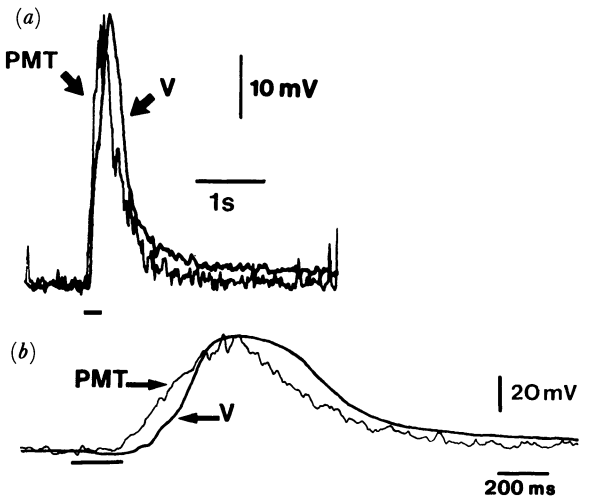

FIGURE 5. (*a*) Aequorin luminescence (trace labelled PMT), indicating a rise in Ca_i, and depolarization (trace labelled V) following the pressure injection into the R-lobe of a *Limulus* ventral photoreceptor of 2 mM calcium aspartate dissolved in 100 mM potassium aspartate, 10 mM HEPES, pH 7.0. The duration of injection is indicated by the bar beneath the traces. The aequorin luminescence is arbitrarily normalized to the peak of the depolarization. For details see Payne *et al.* (1986*a*). (*b*) Aequorin luminescence and depolarization following the pressure injection into a *Limulus* ventral photoreceptor of 1 mM Ins(1,4,5)P_3 dissolved in 100 mM potassium aspartate 10 mM HEPES, pH 7.0. The duration of injection is indicated by the bar beneath the traces. The aequorin luminescence is arbitrarily normalized to the peak of the depolarization.

added calcium, suggesting that calcium is mobilized from intracellular stores (Brown & Rubin 1984; Corson & Fein 1987).

Fractionation of several cell types indicates that Ins(1,4,5)P_3 releases calcium stored in the endoplasmic reticulum (Prentki *et al.* 1984; Streb *et al.* 1984). Given the extensive network of smooth ER that makes up the SMC, it comes as no surprise to find that Ins(1,4,5)P_3 releases

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calcium from the R-lobe of ventral photoreceptors (Brown & Rubin 1984; Payne & Fein 1987*a*). However, an unexpected finding, with an image-intensifier to view aequorin luminescence, is that injections of Ins(1,4,5)P_3 do not release detectable calcium from the light-insensitive A-lobe (Payne & Fein 1987*a*). This result is surprising because the A-lobe contains rough and smooth ER which can utilize ATP to accumulate calcium, as judged by the oxalate precipitation method described above (Walz, unpublished observations). This implies that all ER accumulates calcium, but only ER in the R-lobe is competent to release calcium in response to the injection of Ins(1,4,5)P_3. Several studies of permeabilized cells and preparations of ER have concluded that not all ER is sensitive to Ins(1,4,5)P_3 (Dawson & Irvine 1984; Taylor & Putney 1985; Biden *et al.* 1986). The *Limulus* ventral photoreceptor may represent an extreme example of this differential sensitivity. An obvious hypothesis is that the ER in the A-lobe lacks receptor sites for Ins(1,4,5)P_3 that are present on the SMC. It might be possible to test this hypothesis by autoradiographic localization of the binding of labelled Ins(1,4,5)P_3 within the A- and R-lobes (Worley *et al.* 1987*a*).

The role of calcium release and Ins(1,4,5)P_3 in the electrical response to light

Illumination of *Limulus* ventral photoreceptors increases the electrical conductance of the plasma membrane in the R-lobe, resulting in a flow of current into the cell (see review by Payne 1986). This light-activated current (photocurrent), carried through light-activated ion channels by sodium ions (Millecchia & Mauro 1969; Brown & Mote 1974; Bacigaiupo & Lisman 1983), depolarizes the membrane of the entire cell body. If bright illumination is sustained for several seconds, the photocurrent declines from a peak amplitude of tens or hundreds of nanoamperes to a plateau level of only a few nanoamperes. This decline of the photocurrent is caused by a reduction in the sensitivity to light of the mechanism that opens ionic channels. Thus there are two processes initiated by photo-activated rhodopsin: the generation of the photocurrent, excitation, and the subsequent reduction by adaptation of the ability of light to excite the cell.

Pressure injection of Ins(1,4,5)P_3 into *Limulus* ventral photoreceptors both excites and adapts the photoreceptor (Brown *et al.* 1984; Fein *et al.* 1984). Injections of 1–10 pl of 2–100 μM Ins(1,4,5)P_3 produce bursts of depolarization of amplitude 5–50 mV and each burst of depolarization is associated with a concomitant rise in Ca_i (figure 5*b*) (see also Brown & Rubin 1984; Corson & Fein 1987). The depolarization is caused by the activation of an ionic conductance with similar properties (e.g. reversal potential and permeability to sodium) to that opened by light. Thus it is probable that Ins(1,4,5)P_3 activates the light-sensitive conductance.

Prior injection of calcium chelators abolishes the depolarization caused by injections of Ins(1,4,5)P_3 (Rubin & Brown 1985; Payne *et al.* 1986*b*), suggesting that Ins(1,4,5)P_3 acts solely by releasing calcium and that it has no direct action on the light-sensitive conductance. Consistent with this explanation, pulsed-pressure injection of calcium into the R-lobe activates the light-sensitive conductance (figure 5*a*) (Payne *et al.* 1986*a*). A rise in Ca_i caused by injection of Ins(1,4,5)P_3 or calcium therefore excites the photoreceptor under some conditions.

As well as exciting *Limulus* ventral photoreceptors, injection of Ins(1,4,5)P_3 reversibly inhibits subsequent responses to both diffuse light flashes (Brown *et al.* 1984; Fein *et al.* 1984) and to further injections of Ins(1,4,5)P_3 (Fein *et al.* 1984). This inhibition is analogous to adaptation of the photoreceptors by bright light. The ability of Ins(1,4,5)P_3 to inhibit the

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responses of ventral photoreceptors to light is also abolished by prior injection of calcium chelators, suggesting that, like Ins(1,4,5)P_3-induced excitation, it too arises solely from the release of calcium. We shall consider this inhibitory mechanism in detail in the next section.

The natural breakdown product of Ins(1,4,5)P_3, Ins(1,4)P_2, is less than one tenth as effective as Ins(1,4,5)P_3 in exciting and adapting the photoreceptor (Brown *et al.* 1984; Fein *et al.* 1984), consistent with its lesser ability to release calcium from stores in other cells (see review by Berridge, 1987).

A key, unresolved question is the extent to which production of Ins(1,4,5)P_3 and release of calcium by light mediate excitation and adaptation under normal circumstances. The evidence in favour of calcium as a messenger for adaptation is strong. The essential feature of adaptation, the reduction of the sensitivity of the photocurrent to light, can be mimicked by intracellular injection of calcium (Lisman & Brown 1972) and opposed by the injection of calcium chelators (Lisman & Brown 1975). In addition, the rise in calcium is rapid enough to mediate the 1–2 s time course of the onset of adaptation. Thus calcium released by light is an important factor in mediating light adaptation.

The ability of Ins(1,4,5)P_3 to excite the photoreceptors of invertebrates is not confined to those of the ventral eye of *Limulus*. Introduction of Ins(1,4,5)P_3 into photoreceptors of the lateral compound eye of *Limulus* creates bursts of depolarization similar to those seen in ventral photoreceptors (Payne & Fein 1987*b*). Ins(1,4,5)P_3 has also been introduced into photoreceptors of the housefly compound eye, producing a sustained depolarization made up from the summation of many small events similar to those elicited by single photons (Devary *et al.* 1987). However, the role of the release of Ins(1,4,5)P_3 and the rise in Ca_i in mediating excitation by light is controversial. The injection of calcium chelators does not abolish excitation by light, although it slows the process down (Lisman & Brown 1975). Neither does the rising edge of the light-induced rise in Ca_i, as indicated by aequorin luminescence, seem to precede the generation of the photocurrent (Brown & Blinks 1974; Payne & Fein 1988). Calcium is therefore able to excite the photoreceptor but it may not be the only pathway by which light can open ionic channels. Other pathways for the direct activation of ionic channels may exist, such as a light-induced rise in the concentration of the messenger cyclic GMP (Saibil 1984; Johnson *et al.* 1986). Unfortunately, the lack of knowledge of the degree to which calcium chelators and indicators such as aequorin can rapidly mix with the calcium that light releases into the space between microvilli and sub-rhabdomeral cisternae means that we cannot definitely rule out the possibility that calcium is a messenger of excitation as well as adaptation.

4. The control of calcium release by negative feedback

Light, Ins(1,4,5)P_3 and calcium all inhibit the actions of Ins(1,4,5)P_3

The ability of both Ins(1,4,5)P_3 and light to activate ion channels in the plasma membrane, and so to depolarize *Limulus* ventral photoreceptors, is subject to reversible inhibition by prior bright illumination or by prior injections of Ins(1,4,5)P_3 (Brown *et al.* 1984; Fein *et al.* 1984). With light, this inhibition is part of the adaptation process. If a pair of bright flashes is delivered, the depolarization caused by the second flash is less than that caused by the first. The same is true for injections of Ins(1,4,5)P_3. The second pulsed pressure-

injection of Ins(1,4,5)P_3 in a pair will not depolarize the photoreceptor if it is delivered within 2 s of the first injection (figure 6). Recovery of the response to the second injection takes 10–30 s.

There are several factors that may contribute to the inhibition of the response to Ins(1,4,5)P_3, including a depletion of stored calcium by the first injection of Ins(1,4,5)P_3. Soaking the cells for many minutes in external solutions that contain no calcium, however, does not slow recovery of the response to the second injection of Ins(1,4,5)P_3 (figure 6). Thus if the Ins(1,4,5)P_3-sensitive stores of calcium are depleted after the first injection of Ins(1,4,5)P_3, extracellular calcium is probably not a source of replenishment. If this were so, then removing extracellular calcium would slow or abolish recovery.

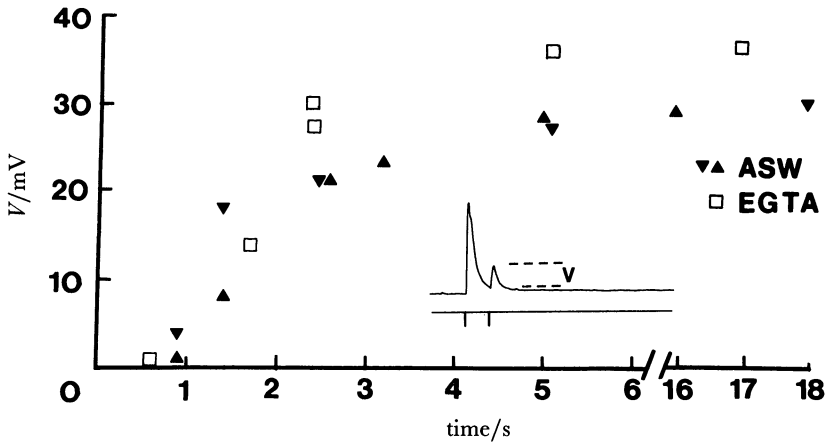

FIGURE 6. The peak amplitude of the response to a second pressure-injection of 100 μM Ins(1,4,5)P_3 into a *Limulus* ventral photoreceptor plotted against the time elapsed after a similar prior injection. Triangles are responses collected while the receptor was bathed in artificial seawater (ASW) containing 10 mM $CaCl_2$. Squares are responses collected after 25 min exposure in darkness to seawater containing 1 mM EGTA and no added calcium. The inset shows an example of the photoreceptor's transmembrane potential during one of the paired injections of 100 μM Ins(1,4,5)P_3. Injection duration was 50 ms, pressure 20 lbf in^{-2} (*ca.* 13.8 kPa). Injections were made into the R-lobe of the photoreceptor. For details of methods see Payne *et al.* (1986*b*).

As both light and the injection of Ins(1,4,5)P_3 have a common ability to raise Ca_i, an interesting possible explanation for the transient inhibition of the response to Ins(1,4,5)P_3 is that elevated Ca_i inhibits the ability of Ins(1,4,5)P_3 to depolarize the photoreceptor. This process would be analogous to the proposed mediation by calcium of adaptation of the depolarization caused by light (Lisman & Brown 1972). To test this possibility, a pulsed pressure-injection of calcium was delivered before an injection of Ins(1,4,5)P_3 and a light flash (figure 7). This injection of calcium reversibly suppressed the ionic current activated by subsequent pulses of Ins(1,4,5)P_3 and by the light flash (figure 7*b*). Thus the activation of ion channels in the plasma membrane by both Ins(1,4,5)P_3 and light is inhibited by raising Ca_i.

The inhibition of the actions of Ins(1,4,5)P_3 may be due to feedback inhibition of Ins(1,4,5)P_3-induced calcium release by elevated Ca_i

As discussed above, we do not yet fully understand the process by which light activates ion channels in the plasma membrane. In addition to releasing calcium, there is evidence that light initiates an unknown mechanism that opens ion channels. Given this uncertainty, we shall not

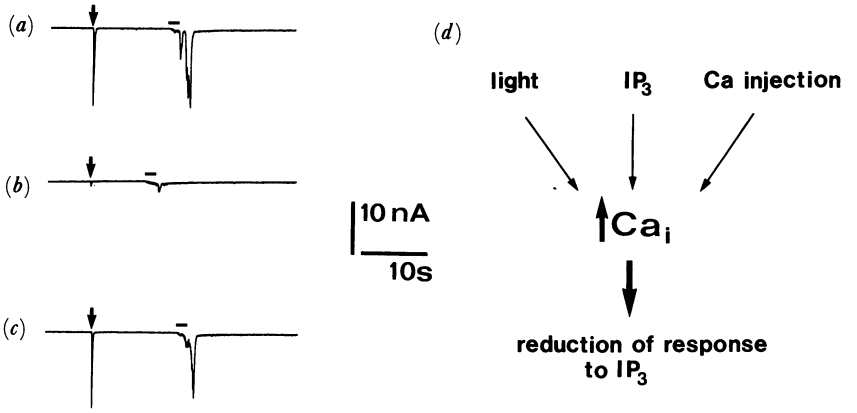

FIGURE 7. (*a–c*) Reversible desensitization by an injection of calcium of responses to light and Ins(1,4,5)P_3. Transmembrane current, recorded with a voltage-clamp, during 10 ms flashes of light (arrows) and 1.5 s intracellular pressure injections of 100 μM Ins(1,4,5)P_3 (bars). Downward deflections indicate that current is flowing into the photoreceptor. The control inward currents elicited by light and Ins(1,4,5)P_3 in (*a*) were strongly inhibited by an injection of 1 mM calcium aspartate delivered 30 s before the record in (*b*). The currents elicited by light and Ins(1,4,5)P_3 recovered to control values 3 min later (*c*). Both Ins(1,4,5)P_3 and calcium aspartate were injected in a carrier solution containing 100 mM potassium aspartate, 10 mM HEPES, pH 7.0. (After Payne *et al.* (1986*a*).) (*d*) Summary of the actions of light flashes, injections of Ins(1,4,5)P_3 and injections of calcium in reducing subsequent responses to Ins(1,4,5)P_3.

speculate about the mechanism by which calcium inhibits depolarization by light during light-adaptation. However, depolarization by Ins(1,4,5)P_3 appears to be solely initiated by the calcium that Ins(1,4,5)P_3 releases, making it possible to investigate the mechanism of its inhibition by prior injections of calcium.

Ins(1,4,5)P_3 does not directly activate ion channels in the plasma membrane but, rather, it is the calcium that is released by Ins(1,4,5)P_3 that activates those channels. There are two possible sites at which elevated Ca_i could antagonize the activation by Ins(1,4,5)P_3 of ion channels in the plasma membrane. Elevated Ca_i could either directly antagonize the opening of channels by the calcium released by Ins(1,4,5)P_3, or antagonize the ability of Ins(1,4,5)P_3 to release calcium. If the first mechanism is active, then the first of a pair of injections of calcium should inhibit the response to the second injection of calcium. However, we have not been able so far to demonstrate such an inhibition acting on depolarization by injections of calcium. The second injection of a pair of pulsed pressure injections of calcium depolarizes the cell to the same extent as the first, even when the two injections are spaced less than 2 s apart. Figure 8 compares responses to paired injections of Ins(1,4,5)P_3 and calcium in two cells, showing the differences in the extent of inhibition of the response to the second injection by the first injection. Neither have we been able to demonstrate substantial inhibition during light-adaptation of the response to injections of calcium (Payne *et al.* 1986*a*). It therefore seems likely that the inhibition of the response to Ins(1,4,5)P_3 by prior injection of Ins(1,4,5)P_3 (figure 6) or calcium (figure 7) is due largely to an inhibition of Ins(1,4,5)P_3-induced release of calcium, rather than an inhibition of the ability of calcium to activate channels in the plasma membrane. This interpretation is supported by direct observation of the greatly reduced rise in Ca_i that accompanies the reduced depolarization caused by the second in a pair of injections of Ins(1,4,5)P_3 (figure 9). We are pursuing experiments to show directly the reduction of the Ins(1,4,5)P_3-induced rise in Ca_i that follows a prior injection of calcium.

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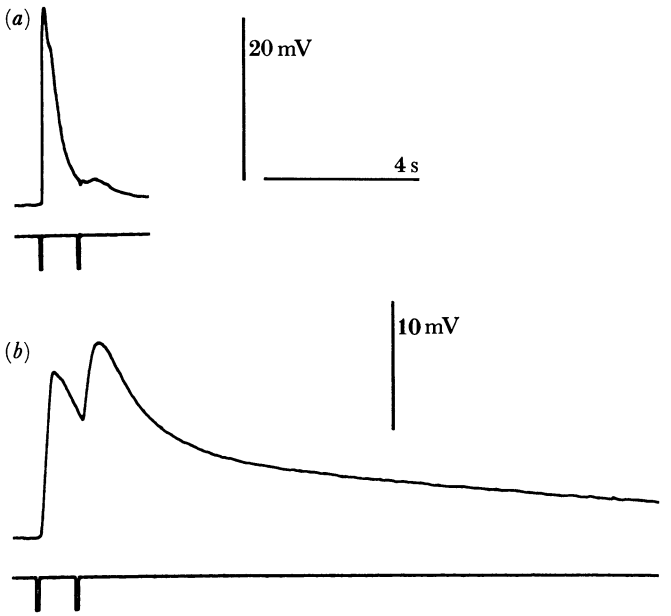

FIGURE 8. (*a*) Depolarization of *Limulus* ventral photoreceptor by paired injections of 100 μM Ins(1,4,5)P_3. The second injection of Ins(1,4,5)P_3 has little effect. The times of injections are indicated by the bottom trace. Same cell as in figure 7. (*b*) Depolarization of another *Limulus* ventral photoreceptor by paired injections of 1 mM calcium aspartate The second injection restores the depolarization elicited by the first injection to its peak amplitude. For details of injection methods see Payne *et al.* (1986*a*).

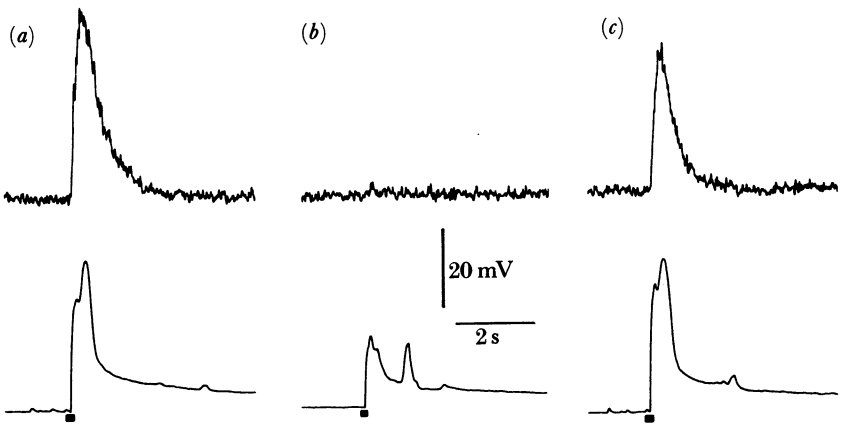

FIGURE 9. Injections of 1 mM Ins(1,4,5)P_3 into a *Limulus* ventral photoreceptor. Upper traces indicate aequorin luminescence elicited by the injections, showing elevated Ca_i. Lower traces indicate transmembrane potential, showing simultaneous depolarizations. A control injection of Ins(1,4,5)P_3 (*a*) was followed by a second injection 30 s later (*b*). The second injection elicited a smaller (adapted) depolarization. The corresponding PMT record of (*b*) indicates that the rise in Ca_i as monitored by aequorin was much smaller. A third injection 3 min later (*c*) elicited a depolarization and aequorin luminescence similar to those in (*a*). The injection duration is indicated by the bars beneath the lower traces. (After Payne *et al.* (1986*b*).)

Negative feedback control of calcium release may be a mechanism of sensory adaptation

We propose the model of figure 10 to explain the actions of Ins(1,4,5)P_3 in *Limulus* ventral photoreceptors. Ins(1,4,5)P_3 specifically releases calcium from the SMC of smooth endoplasmic reticulum beneath the plasma membrane. The released calcium has two functions. Firstly, it opens ionic channels in the plasma membrane, either directly by binding

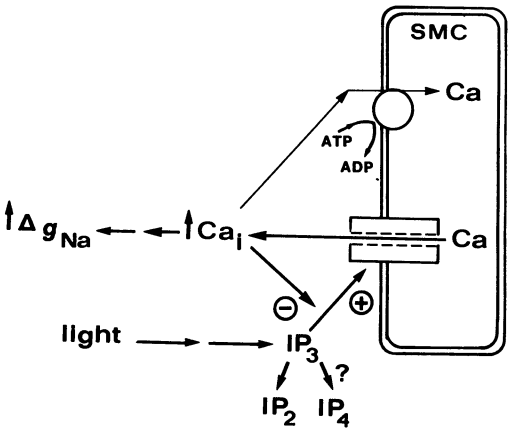

FIGURE 10. Model of the release of calcium from the SMC by Ins(1,4,5)P_3 (IP_3) in *Limulus* ventral photoreceptors. The membrane of the SMC contains a calcium pump that utilizes ATP to sequester calcium and a calcium pore that is opened by Ins(1,4,5)P_3 to release calcium into the cytosol. The released calcium then increases the sodium permeability of the plasma membrane (Δg_{Na}) and feeds back to inhibit the action of Ins(1,4,5)P_3 on the calcium pore in the SMC. Ins(1,4,5)P_3 is metabolized to Ins(1,4)P_2 (IP_2) and possibly to Ins(1,3,4,5)P_4 (IP_4).

to the channels or through the release of another messenger. Secondly, it feeds back to inhibit further calcium release by Ins(1,4,5)P_3.

Recent studies on some types of permeabilized cells show that micromolar free calcium can inhibit calcium release from intracellular stores by Ins(1,4,5)P_3 (Suematsu *et al.* 1984; Chueh & Gill 1986; Thierry & Klee 1986). Also, binding of Ins(1,4,5)P_3 to homogenized neural tissue appears to be inhibited by micromolar free calcium (Worley *et al.* 1987). Our proposal of feedback inhibition in living *Limulus* ventral photoreceptors therefore reflects what may be a general attribute of Ins(1,4,5)P_3-induced calcium release. More importantly, we can begin to explore the physiological consequences of this control.

The most important consequence of the negative feedback control of calcium release is that it may enable adaptation of the release of calcium by light. If bright illumination of a *Limulus* ventral photoreceptor is sustained for several seconds, the free calcium concentration in the R-lobe declines from its initial peak to a plateau value of less than 5 μM (figure 11). This decline in Ca_i accompanies the decline in the photocurrent and the depolarization of the cell during adaptation to light. An alternative view of the same adaptational process can be obtained by delivering pairs of bright flashes of light with a variable time between them. The rise in Ca_i caused by the second flash is greatly reduced if the interval between flashes is short, recovering if the interval is extended to a few minutes (Brown & Blinks 1974; Maaz & Stieve 1980; Levy & Fein 1985). During this interval the level of Ca_i recovers from its elevation by the first flash (Nagy & Stieve 1983; Levy & Fein 1985). The slow decline of Ca_i after an adapting light can also be seen in figure 11.

The adaptation of the light-induced rise in Ca_i may have several possible causes. The ability of light to release calcium may be inhibited, the stores of calcium may be depleted or the buffering and pumping systems that lower Ca_i may be stimulated. Also, any (small) component of the rise in Ca_i that is caused by calcium influx through the light-sensitive ion channels in the plasma membrane (Maaz & Stieve 1980; Nagy & Stieve 1983) or through channels in the plasma membrane activated by depolarization (O'Day *et al.* 1982) will be suppressed by the reduction by light-adaptation of the number of channels opening after a

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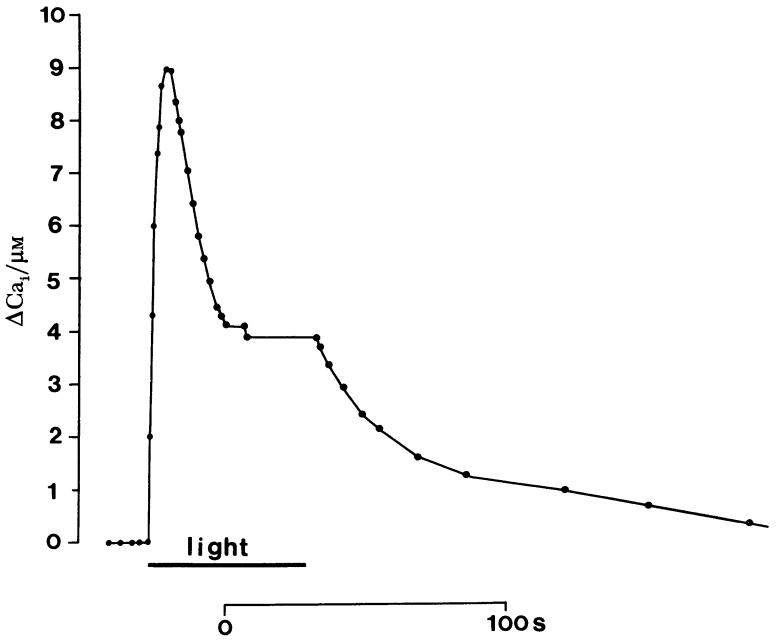

FIGURE 11. Cytosolic free calcium concentration, recorded during a prolonged light flash (bar) using a calcium-sensitive microelectrode placed in the R-lobe of a *Limulus* ventral photoreceptor. The initial peak of Ca_i, immediately after the onset of light, is probably underestimated owing to the slow response time of the calcium electrode. After the cessation of light, Ca_i slowly declines towards its initial value in darkness. The trace is the conversion of the electrode potential into calcium concentration after digitization of the original chart-record of electrode potential by hand. For details see Levy & Fein (1985).

flash. The relative contributions of these factors in reducing Ca_i during prolonged illumination is impossible to assess, given the lack of quantitative information about calcium metabolism in these photoreceptors. However, if calcium release by light is indeed mediated by the production of Ins(1,4,5)P_3 then it is possible that, after a bright flash of light, the elevated Ca_i inhibits calcium release by subsequent light flashes.

If elevated Ca_i initiates the inhibition of light-induced calcium release that follows a light flash, then to what extent does the decline in Ca_i during the recovery from adaptation mediate the recovery of light-induced calcium release? Nagy & Stieve (1983) investigated calcium release by pairs of bright flashes and found that an initial, rapid phase of the recovery of the ability of a second flash to release calcium occurs as resting levels of Ca_i decline. A second phase of recovery, however, occurs after resting levels of Ca_i have returned close to their dark-adapted value (see also Levy & Fein 1985). Whether this second phase represents a lingering effect of elevated Ca_i or another mechanism of adaptation is not known. Also, as the relationship between sensitivity and elevated Ca_i appears to be very steep (Levy & Fein 1985), it is possible that the methods of measurement of Ca_i were not sensitive enough to detect a small elevation of Ca_i close to the sites that control calcium release.

During prolonged illumination, adaptation avoids saturation of the sites that respond to calcium and prevents complete discharge of the calcium stores. For other cell types, adaptation may or may not be a desirable feature of the calcium-release mechanism, depending on the function of the cell. Adaptation is clearly more desirable in cells responding transiently to temporal changes in stimulus rather than in a sustained manner to a sustained stimulus.

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The feedback control may rapidly terminate responses to brief injections of Ins(1,4,5)P_3 and create oscillatory responses to large injections

A second consequence of feedback control is that it may operate so rapidly that desensitization, rather than metabolism or dilution of Ins(1,4,5)P_3, terminates the response to brief pulsed injections of Ins(1,4,5)P_3 delivered directly into the R-lobe. As can be seen in figure 9, desensitization is already maximal at the base of the depolarization caused by Ins(1,4,5)P_3, so that it is conceivable that desensitization of calcium release causes the depolarization to decline and stops further calcium release until metabolism and dilution of Ins(1,4,5)P_3 have reduced the local concentration of Ins(1,4,5)P_3 below threshold.

After large injections of Ins(1,4,5)P_3, the membrane potential of the photoreceptor oscillates, indicating oscillatory bursts of calcium release (figure 12*c*). Oscillations of depolarization are not observed after injections of calcium, suggesting that the oscillations originate in the release of calcium by Ins(1,4,5)P_3 rather than in the response to released

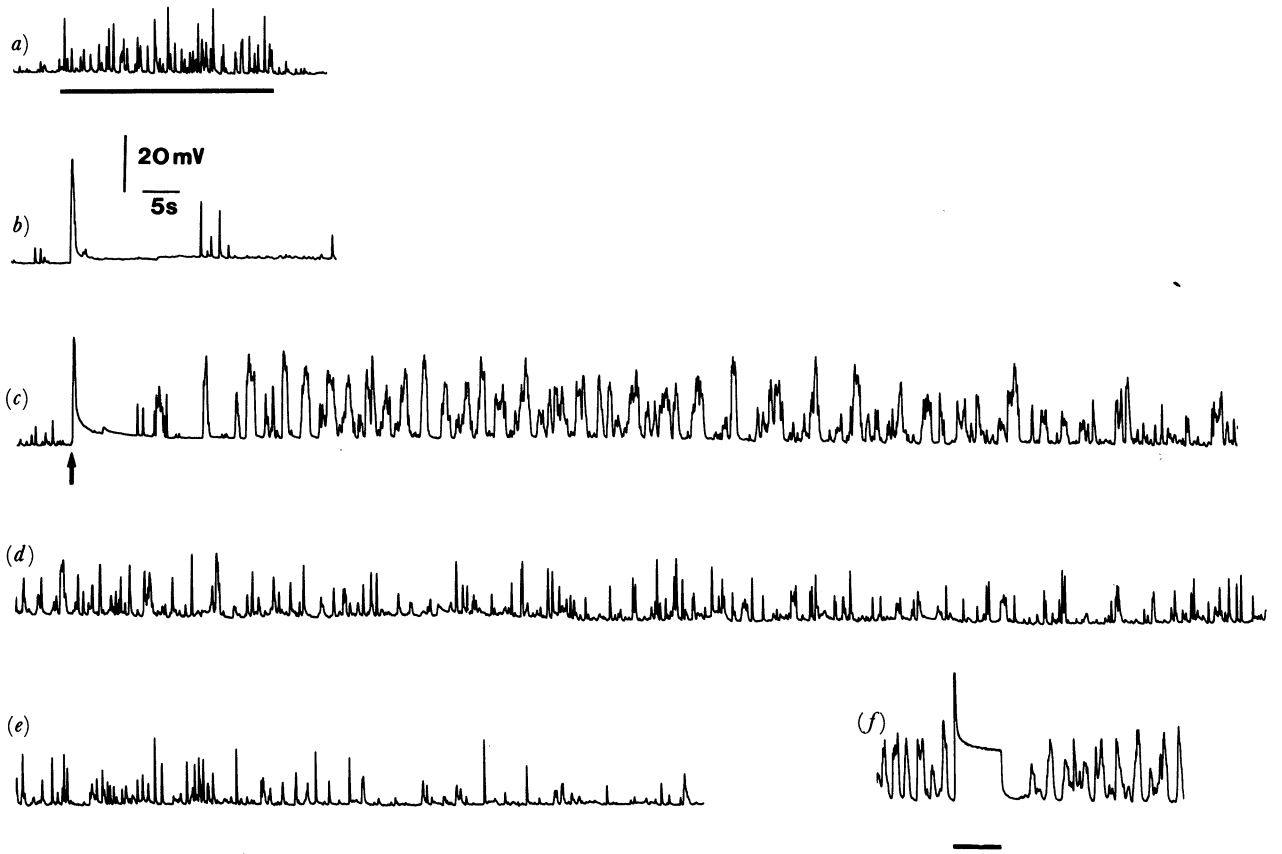

FIGURE 12. (*a*) Individual depolarizing events ('quantum bumps') produced by very dim illumination (bar) of a *Limulus* ventral photoreceptor. Each quantum bump is thought to result from a single photoisomerization of rhodopsin (Yeandle & Spiegler 1973). (*b*) A single 100 ms pressure-injection of 100 μM Ins(1,4,5)P_3 produces a single smooth transient depolarization. (*c–e*) A subsequent 300 ms injection of 100 μM Ins(1,4,5)P_3 causes a single smooth transient depolarization, followed by a 'silent period' of desensitization and then a series of oscillatory bursts of transient depolarization that eventually break up (*d*, *e*) into events resembling those produced by single quanta. (*f*) The oscillations of depolarization caused by another 300 ms injection of Ins(1,4,5)P_3 into the same cell can be transiently suppressed by bright illumination (bar), indicating that they originate in the light-, Ins(1,4,5)P_3- and calcium-sensitive mechanism that governs calcium release.

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calcium. The oscillations eventually break up into a series of discrete depolarizing events that resemble events caused by single photons of light (compare figure 12*d*, *e* with figure 12*a*).

The oscillations in depolarization may be explained by the feedback model of figure 10. If the Ins(1,4,5)P_3 concentration after a large injection is still above threshold when the 2–10 s period of desensitization is over, then a new cycle of calcium release, depolarization and desensitization may be initiated. The period of oscillation varies from cell to cell, but the range, 3–10 s, is similar to the delay before sensitivity recovers after the release of calcium (see, for example, figure 6). Other factors such as reloading of the calcium stores and local fluctuations in Ins(1,4,5)P_3 concentration, however, may also contribute to what is obviously a very complex response to Ins(1,4,5)P_3.

The use of feedback models to explain oscillations in Ca_i has been investigated by Rapp & Berridge (1977), although the mediator of the inhibition of calcium release in their models, cyclic AMP, is not a strong candidate as a messenger of excitation or adaptation of phototransduction in *Limulus* photoreceptors (see review by Payne 1986). We therefore favour a more direct action of Ca_i on the calcium-release mechanism of the SMC.

Oscillations in free calcium have been observed after injections of Ins(1,4,5)P_3 into *Xenopus* oocytes (Parker & Miledi 1986; Ferguson *et al.* 1987) and during constant intracellular dialysis of hepatocytes with Ins(1,4,5)P_3 with the whole cell patch technique (Capiod *et al.* 1987; also see review by Berridge 1987). These oscillations may constitute part of the normal response to hormonal stimuli in hepatocytes (Woods *et al.* 1986). Light-induced oscillations in Ca_i have never been reported in the *Limulus* photoreceptor, although large bursts of depolarization are sometimes seen in darkness after very bright flashes (Fein & Hanani 1978; Corson *et al.* 1983). Large injections of Ins(1,4,5)P_3 also sometimes elicit isolated bursts of depolarization, each burst being accompanied by a rise in Ca_i (Corson & Fein 1987). It may be that a photon of light releases Ins(1,4,5)P_3 into a localized section of the 100 nm space between the plasma membrane and the SMC. Efficient coupling of Ins(1,4,5)P_3 in that space to the Ins(1,4,5)P_3-sensitive receptors that release calcium, together with efficient hydrolysis of Ins(1,4,5)P_3, may prevent a build-up or spread of Ins(1,4,5)P_3 similar to that associated with the large injections of Ins(1,4,5)P_3 that create oscillations and bursts of calcium release. Light-induced release of calcium is confined to the region of the R-lobe that is illuminated (Fein & Payne 1988), suggesting efficient local metabolism of the Ins(1,4,5)P_3 released by light. Intriguingly, the oscillations in figure 12 eventually break up into events resembling single-photon events, suggesting that the normal and oscillatory response are closely related. Alternatively, light may release other messengers, in addition to Ins(1,4,5)P_3, that may damp the oscillations or accelerate the hydrolysis of Ins(1,4,5)P_3 in the vicinity of a photoisomerization.

Conclusion

Invertebrate photoreceptors seem to contain the machinery needed to produce and respond to Ins(1,4,5)P_3. Whether or not this signalling pathway is sufficient to account entirely for excitation and adaptation of the cell by light remains to be seen. There is, for instance, preliminary evidence for the involvement also of cyclic GMP in excitation (Saibil 1984; Johnson *et al.* 1986). However, the control of the phosphoinositide pathway alone provides a very complex problem, with feedback controls at several levels. 'Upstream' from the production of Ins(1,4,5)P_3, phosphorylation of metarhodopsin may turn off activation of the

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phosphoinositide cascade. 'Downstream' from Ins(1,4,5)P_3 production, a powerful feedback mechanism limits the release of calcium and may shape the response to injected Ins(1,4,5)P_3 into a series of local transient rises in Ca_i. The complexity of the control of the release of calcium is further increased by the localization of calcium stores within the cell. The close apposition of structures that produce and respond to Ins(1,4,5)P_3, coupled with the generation of very large local transient rises in Ca_i, results in a precise trigger for intracellular signalling.

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FIGURE 3. For description see opposite.

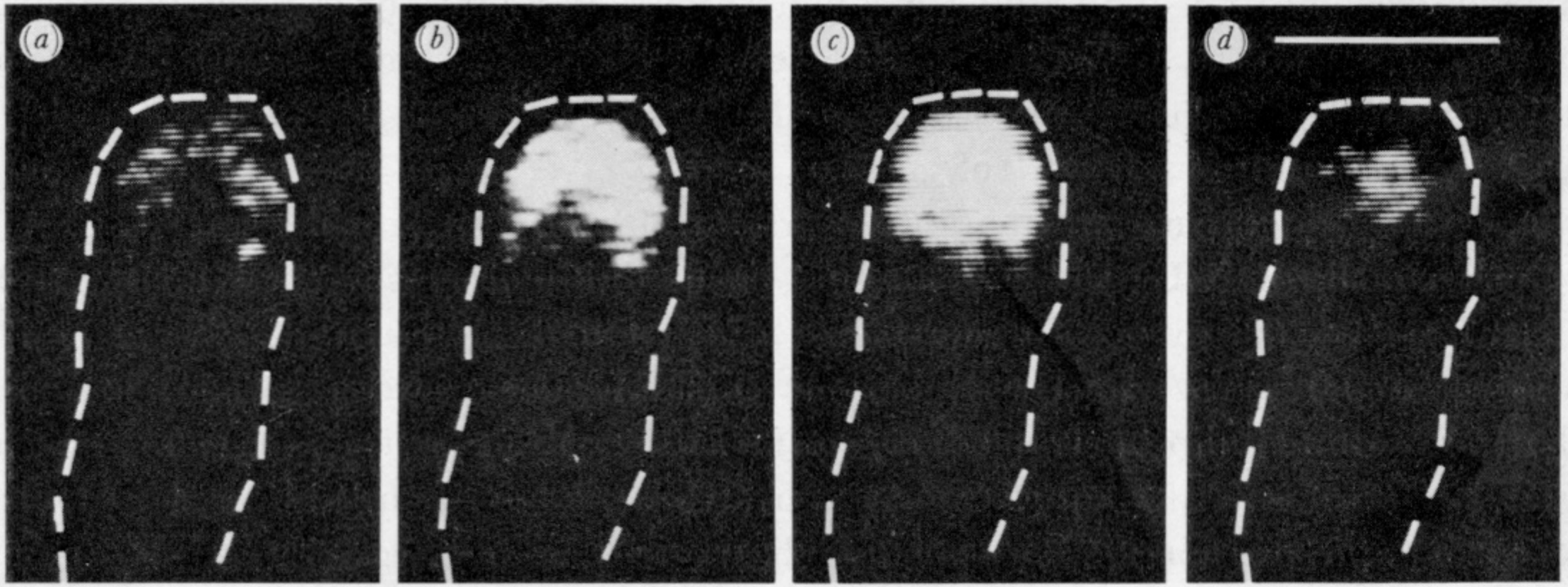

FIGURE 4. Images of aequorin luminescence taken at increasing times after a 10 ms flash was delivered to a *Limulus* ventral photoreceptor. The diffuse flash illuminated the entire field of view. The broken line outlines the cell body, with the axon exiting from the arhabdomeral lobe towards the bottom of each video frame and the rhabdomeral lobe situated towards the top of each frame. Aequorin luminescence is always confined to the distal region of the cell in the R-lobe. Each image is a single video frame, the frames being recorded 230 ms (*a*), 460 ms (*b*), 1160 ms (*c*) and 3000 ms (*d*) after the end of the light flash. Aequorin luminescence begins at the periphery of the R-lobe (*a*), spreads to fill in the centre of the R-lobe (*b–c*) and declines first at the periphery of the R-lobe (*d*). The images were taken with a Nikon M-Plan 20 × N.A. 0.4 microscope objective focused on to the photocathode of a Venus Scientific Instruments TV3M image intensifier. The video output of the intensifier was stored on the video disk of a motion analyser (Sony model SVM-1010) and individual frames were photographed. Scale bar, 50 μm. For further details see Payne & Fein (1987*a*).

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