Fast desensitization of the response to $InsP_3$ in *Limulus* ventral photoreceptors

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ABSTRACT In *Limulus* ventral photoreceptor cells the time-course of the desensitization of $InsP_3$ response was measured by an injection-pair paradigm. Pressure pulses of $InsP_3$ were delivered into the cell with various interpulse intervals. The desensitization of the response to the second injection of each pair approached totality at 200 ms, which is the duration of the response to a single pressure pulse of $InsP_3$. Lowering extracellular calcium did not affect the time-course of the desensitization. Lowering the temperature slowed down both the time-course of the response to $InsP_3$ and the time-course of the desensitization to the same extent. These findings suggest that the desensitization is powerful enough and its onset fast enough to contribute to the transience of the $InsP_3$ response. The time-course of the desensitization suggests it may influence light adaptation.

INTRODUCTION

D-myo-inositol 1,4,5-trisphosphate ($InsP_3$) has been shown both to excite and to adapt *Limulus* ventral photoreceptors (Brown et al., 1984; Fein et al., 1984; Payne et al., 1986*b*).

A brief (20–200 ms) injection of $InsP_3$ into the light-sensitive lobe of the photoreceptor cell produces a transient depolarization of the cell membrane lasting 100 ms–1 s and a prolonged desensitization (Brown et al., 1984; Fein et al., 1984; Payne et al., 1986*b*). An injection of a metabolically stable analogue of $InsP_3$, DL-myo-inositol 1,4,5-trisphosphorothioate ($InsP_3S_3$), produces a large transient depolarization similar to that caused by $InsP_3$ and followed by a period of insensitivity to further injections of $InsP_3S_3$. As sensitivity returns, a series of oscillatory depolarizing bursts begins and continues for at least tens of minutes (Payne and Potter, 1991). The failure of a metabolically-stable analogue to produce a smooth depolarization suggests that a fast desensitization mechanism must be involved in terminating both the initial transient response and individual depolarizing bursts (Payne and Potter, 1991). Payne et al. (1990) delineated the time-course of the recovery from the desensitization, which lasts 15–20 s, but only set an upper limit of 1 s on the onset time of the desensitization.

An injection of $InsP_3$ into the photoreceptor cell produces a transient elevation of the intracellular Ca^{2+} that depolarizes the cell membrane and desensitizes the cell to further injections of $InsP_3$ (Brown and Rubin, 1984; Payne et al., 1986*a*; Payne et al., 1986*b*). Payne et al. (1990) showed that the desensitization of the cell results from feedback inhibition of calcium release by elevated Ca^{2+} levels (Payne et al., 1990). Inhibition of $InsP_3$-induced Ca^{2+} release by Ca^{2+} has been demonstrated in a variety of other tissues (Jean and Klee, 1986; Worley et al., 1987; Joseph et al., 1989; Parker and Ivorra, 1990). Activation of $InsP_3$-sensitive Ca^{2+} channels incorporated into planar phospholipid bilayers also is inhibited by a high concentration of Ca^{2+} applied to the cytosolic side of a channel (Suarez-Isla et al., 1991; Bezprozvanny et al., 1991).

Elevation of Ca^{2+} is probably necessary and sufficient also for the desensitization of the light-induced depolarization that constitutes light adaptation (Lisman and Brown, 1972; Brown and Blinks, 1974). $InsP_3$ has been proposed as a possible mediator of the light-induced depolarization (Brown et al., 1984; Fein et al., 1984) and it is of interest to determine whether feedback inhibition of the response to $InsP_3$ is as fast as or faster than the onset of desensitization in light adaptation.

In this paper we show that the time-course of the desensitization of the $InsP_3$ response by the response to a preceding injection of $InsP_3$ is indeed fast enough to account for the transience of the response to $InsP_3$. The time-course of the onset of the desensitization is also compatible with the time-course of the onset of light adaptation in the ventral photoreceptor.

MATERIALS AND METHODS

The morphology of the *Limulus* ventral photoreceptor is well known (Calman and Chamberlain, 1982) and the methods for its isolation have been described (Clark et al., 1969). In the present work the nerves were dissected out, and their enclosing blood vessels were removed. The connecting tissue remaining in the nerves was digested with 0.7–0.9% pronase (Calbiochem, San Diego, California). After this treatment, the nerves were washed and pinned into a plexiglass chamber, volume 0.5 ml, and artificial sea water (ASW) was passed through the chamber at a rate 5 ml/min. For the low temperature experiments the ASW was cooled with a Peltier device (Interconnection Products Inc., Pompano Beach, Florida) prior to entering the chamber. A digital thermometer (model DP30; Omega Engineering Inc., Stamford, Connecticut) was used to record the temperature in the ASW as sensed by a miniature copper-constantan thermocouple placed in the chamber.

Intracellular recordings were performed with an Axoclamp amplifier (Axon Instruments Inc., Burlingame, California). For current recordings photoreceptors were clamped at their resting (dark) potentials by a conventional two-electrode voltage-clamp (Katz and Schwartz, 1974; Smith et al., 1980). The photoreceptors were impaled with two micro-

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pipettes. An injection micropipette filled with $InsP_3$-carrier solution was used as a voltage electrode and a micropipette filled with 2 M KCl as a current electrode. Clamp current was measured by a current-to-voltage converter in a virtual ground circuit. In voltage clamp the current was passed through a two-pole filter, corner frequency 100 Hz. During voltage clamp the cell can be considered isopotential (Brown et al., 1979).

Conventional methods for light stimulation were used, similar to those described by Fein and Charlton (1977). Rapid pressure injection of substances into cells through single-barreled micropipettes was achieved as described by Corson and Fein (1983). The electronic valve that switched pressure to the micropipette was placed 5 cm from the micropipette holder. A miniature pressure transducer (model PX-176-100S-5V; Omega Engineering Inc.) was inserted between the valve and the micropipette holder, linked to both by a polyethylene tube, as described by Payne and Flores (1992).

$InsP_3$ was obtained from Calbiochem (San Diego, California) as the trilithium salt. Prior to injection it was dissolved in a carrier solution (100 mM potassium aspartate, 10 mM Hepes pH 7.0). EGTA and potassium aspartate were obtained from Sigma Chemical Co. (St. Louis, Missouri). Cells were normally bathed in ASW containing 435 mM NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 25 mM $MgSO_4$, and 10 mM Hepes, pH 7.0. Low Ca^{2+}-ASW was made by replacing the $CaCl_2$ with 1 mM EGTA.

Pairs of brief (10–50 ms) pressure pulses were applied to the pipette containing $InsP_3$ (10–100 μM), which impaled the photoreceptor's light-sensitive R-lobe. Payne and Fein (1987) showed that injections of $InsP_3$ into the R-lobe cause a depolarization response whereas injections into the A-lobe do not. In our experiments the position of the pipette in the R-lobe was identified initially by sight and then confirmed by the fact that the injection resulted in a response. The time-interval between the pressure pulses varied between 40 ms and 60 s measured from the beginning of the first injection to the beginning of the second.

RESULTS

Fig. 1 shows a typical pattern of responses to double injections of $InsP_3$ in a cell bathed in normal Ca^{2+}-ASW at room temperature (20–22°C). The figure shows the inward current resulting from a single injection and from pairs of injections separated by various intervals. With a 40 ms interval (Fig. 1 *b*) the total response area for a pair of injections is roughly double that for a single injection (Fig. 1 *a*). We refer to total area because the responses are not separable here. At an interval of 100 ms the total area is significantly reduced (Fig. 1 *c*) while at 200 ms, when the response to the first injection has reached the baseline, the second response is completely abolished (Fig. 1 *d*). The response to the second injection is still absent at an interval of 1 s (Fig. 1 *e*) and gradually recovers over 5–20 s (Fig. 1 *f*). The time-course of the recovery has been measured previously by Payne et al. (1990) and these observations are consistent with their results.

The desensitization affects not only the total area of the response to the subsequent injection, but also its time-course. The time between the peak of the injection and the peak of the response is significantly longer for the second injection than for the first (Fig. 1, *b* and *c*). This observation was consistent over repeated injections in different cells and from cell to cell.

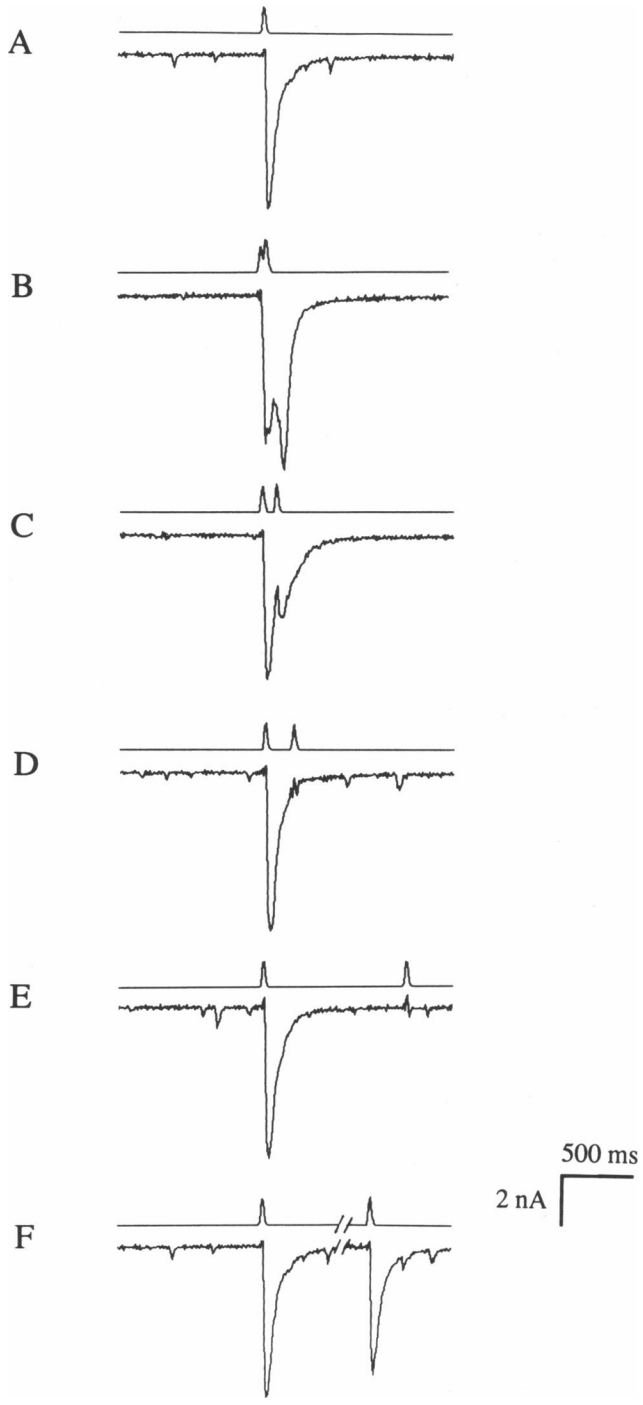

FIGURE 1 Injection of $InsP_3$ into *Limulus* ventral photoreceptors inhibits the responses to subsequent $InsP_3$ injections. The figure shows recordings of the voltage clamp current during paired injections of 100 μM $InsP_3$ at room temperature (19–21°C). Small inward currents before or after the response are quantum bumps, which may be spontaneous or may result from stray light. Upper traces show the pressure developed at the electrode holder as sensed by a pressure transducer. Duration of the injections: 20 ms; pressure: 40 psi. (*a*) Single injection; (*b–e*) pairs of $InsP_3$ injections delivered by the same electrode with intervals between the onset of the two injections of 40, 100, 200 ms and 1 s, respectively; (*f*) two injections with an interval of 20 s between them. In this case the time-scale is not continuous.

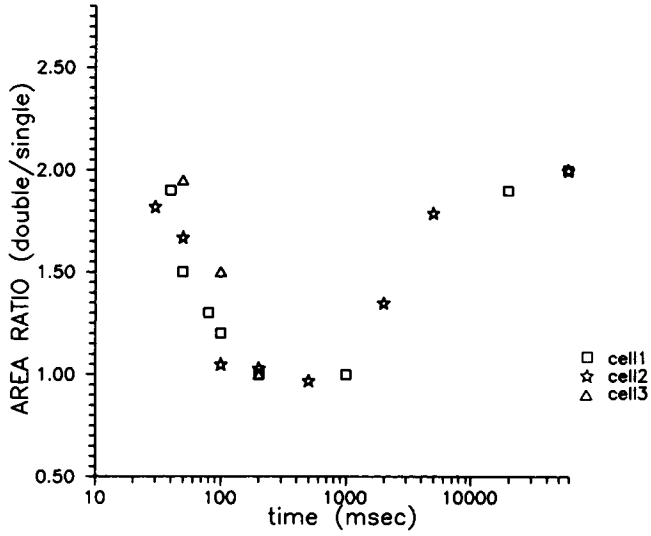

FIGURE 2 The time-course of the desensitization of the $InsP_3$ response. At short time-intervals (less than 200 ms between the injections) the responses to the first and to the second injections overlap. For this reason we measured the total area of the response to the pair of injections and as a measure of the desensitization calculated ratios between an average area of the response to the pair of injections at a given time-interval and an average area of the response to the single injections. Cell 1: 100 μM $InsP_3$, 20 ms injection duration, 40 psi pressure (examples from this cell are shown in Fig. 1). Cell 2, cell 3: 20 μM $InsP_3$, 20 ms duration, 30 psi.

Fig. 2 shows the dependence on inter-injection interval of the area of the current response to double injections divided by the area of response to a single injection into the same cell by the same electrode. A ratio of 2 corresponds to absence of a desensitizing effect, while a ratio of 1 indicates complete desensitization.

Depolarizations measured without application of a voltage clamp in seven cells gave quite similar results (except for an initial ratio above 2; such a ratio may well arise from a voltage-dependent conductance). This similarity enabled us to rely on measurements of depolarizations, which are more easily performed in testing the dependence of the desensitization time-course on extracellular calcium and temperature.

Fig. 3 compares the time-course of the desensitization for cells bathed in normal ASW (average of 6 cells) and for cells bathed in 0-Ca^{2+}-ASW (average of 3 other cells). Payne et al. (1988) showed that removing the extracellular Ca^{2+} has no effect on the time-course of the recovery from the desensitization. Fig. 3 shows that the onset of the desensitization is also unaffected by Ca^{2+} influx from the extracellular space.

In 2 cells (out of 15), desensitization was incomplete. Despite this, the $InsP_3$ response was not prolonged and instead the shape of the response to the first injection was similar to those in all the other cells.

In an attempt to uncouple the time-course of the desensitization from that of the response we lowered the temperature of the bath solution to 10–13°C. This reversibly caused the disappearance of the response to the injection of $InsP_3$ in most of the cells (3 out of 4 cells), while only somewhat reducing and slowing the responses to brief flashes of dim light (5 log of attenuation). In the absence of extracellular calcium the response to $InsP_3$ no longer disappeared at low temperatures (3 cells). The desensitization observed at low temperatures might, therefore, have been caused by a reduced activity of Ca^{2+} pumps at low temperatures, so that a localized leak of calcium into the cell at the site of the electrode penetration caused the desensitization of the response to $InsP_3$ injection.

In 0-Ca^{2+}-ASW at low temperature, the area of the response to $InsP_3$ was increased and its time-course was slowed. These effects were reversible. The observations are illustrated in Fig. 4, which shows responses to $InsP_3$ injections into a cell bathed in 0-Ca^{2+}-ASW solution as the bath was cycled twice between room and low temperature. The right column of the figure shows responses to single (Fig. 4 *g*) and double (Fig. 4, *h–l*) injections of $InsP_3$ when the temperature of the extracellular medium was 13°C. The left column shows the responses of the same cell to single (Fig. 4 *a*) and double (Fig. 4, *b–f*) injections when the temperature was increased to 20°C and the nerve recovered from the low temperature treatment. In both cases at short time-intervals between the injections (50 ms) the area of the response to a pair of injections is roughly double that for the single injection. At longer time-intervals the response to the second injection decreases. The decline in the response to the second injection is more rapid at 20° than at 13°C.

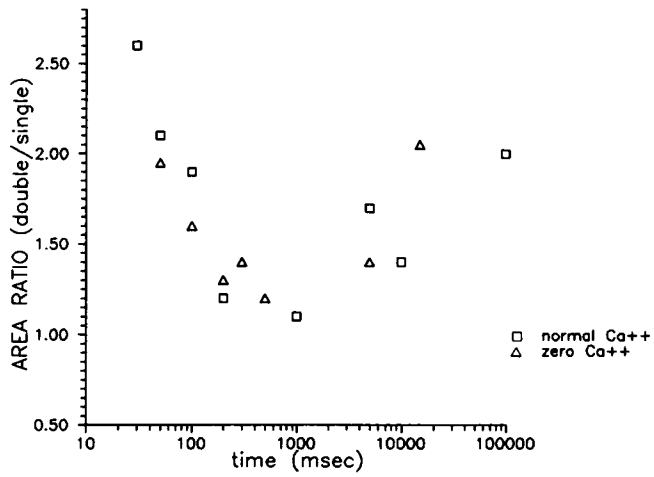

FIGURE 3 Dependence of the time-course of the desensitization of the $InsP_3$ response on extracellular Ca^{2+}. The points represent averages of 6 cells bathed in 10 mM Ca^{2+}-ASW (high Ca^{2+}) and of 3 other cells bathed in 0 Ca^{2+}, 1 mM EGTA ASW (low Ca^{2+}). Records for a given time-interval were averaged separately for each cell and ratios between responses to double injections and single injections were calculated. These ratios were then averaged over the cells. $InsP_3$ concentrations were 10–100 μM, duration 10–50 ms.

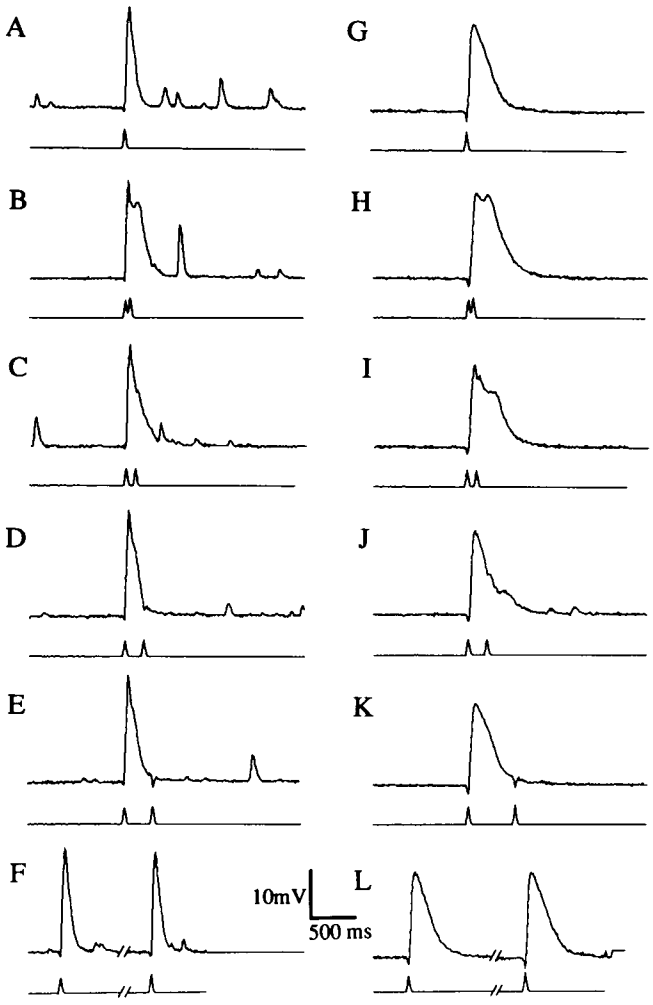

FIGURE 4 Lowering the temperature slows down both the time-course of the response to $InsP_3$ and the desensitization of the $InsP_3$ response by a prior injection. Upper traces show membrane potentials resulting from paired injections of 100 μM $InsP_3$ delivered with short time-intervals between them. Lower traces show pressure developed at the electrode holder, duration of the injection 20 ms, pressure 30 psi. (*a–f*) 20°C; (*g–l*) 13°C. Traces *a–f* were recorded after traces *h–g* when the cell had recovered from the reduced temperature. (*a* and *g*) Single injections; (*b* and *h*) 50 ms interval; (*c* and *i*) 100 ms; (*d* and *j*) 200 ms; (*e*) 300 ms; and (*k*) 500 ms interval. Traces *f* and *l* show the recovery of the response to the second injection, the interval between the injections in this case is 60 s. Differences in the desensitization time-courses are most obvious at intervals 100–200 ms. The experiment was performed at 0-Ca^{2+}-ASW.

Fig. 5 graphs the effect on the time-course of the desensitization of changing the temperature from 19–21° to 11–13°C (average of three cells, all bathed in 0-Ca^{2+}-ASW). In one of these three cells the desensitization was incomplete. The minimum response ratio in Fig. 5 is therefore greater than 1. Records at room temperature were taken before and after records at low temperature. The inserts in Fig. 5 show a comparison of the time-courses of the depolarization responses at high and low temperatures and of the time-courses of the desensitization at these two temperatures in the same cell. As may be seen in the inserts and in Fig. 4 the desensitization appears to be complete as soon as the second injection falls after the end of the response to the first at both temperatures. Fig. 5 confirms this observation by showing that the desensitization approaches its maximum at ~200 and 500 ms at room and low temperatures, respectively. These are also the durations of the responses to single injections at these two temperatures as may be seen in the inserts in Fig. 5. This observation provides further evidence that the desensitization plays a role in the transience of the $InsP_3$ response.

DISCUSSION

The response to an $InsP_3$ injection is transient, with a fast rise and fall. The time-course of the initial response to a non-hydrolyzable analogue of $InsP_3$ is similar, suggesting that hydrolysis of $InsP_3$ is not responsible for the rapid decline of the response (Payne and Potter, 1991). The termination of the response must therefore be due either to the reduction of $InsP_3$ concentration in the $InsP_3$-sensitive portion of the cell by diffusion or to the reduction of its effectiveness in releasing Ca^{2+} from intracellular stores.

The main aim of this work was to measure directly if the time-course of the desensitization of $InsP_3$ effectiveness is fast and powerful enough to terminate the $InsP_3$-induced response in *Limulus* ventral photoreceptors. The fast onset of the desensitization as shown in Figs. 1 and 2 suggests that the reduction in $InsP_3$ effectiveness is indeed fast enough to contribute to the transience of the $InsP_3$ response. The temperature dependence of the onset of the desensitization was measured in an attempt to uncouple the time-course of the desensitization from that of the response. If diffusion of $InsP_3$ is responsible for the declining phase of the response rather than desensitization, the time-course of single responses might be influenced less than that of desensitization and two processes might be separable. In Figs. 4 and 5 we see that lowering the temperature did not uncouple the two time-courses, in agreement with the idea that desensitization and not diffusion causes the termination of $InsP_3$ response. However, in those 2 cells (out of 15) in which the desensitization was incomplete the time-course of the response was not longer than in the other cells, suggesting that either hydrolysis or diffusion or both may be also fast enough to contribute to the termination of $InsP_3$ response.

The observations in the figures appear to suggest a delay in the onset of desensitization. We are however reluctant to interpret this delay in terms of the mechanism of desensitization because of the possibility that at short inter-injection intervals the material from the second injection may physically displace that from the first, thereby simply enlarging the directly affected area. This

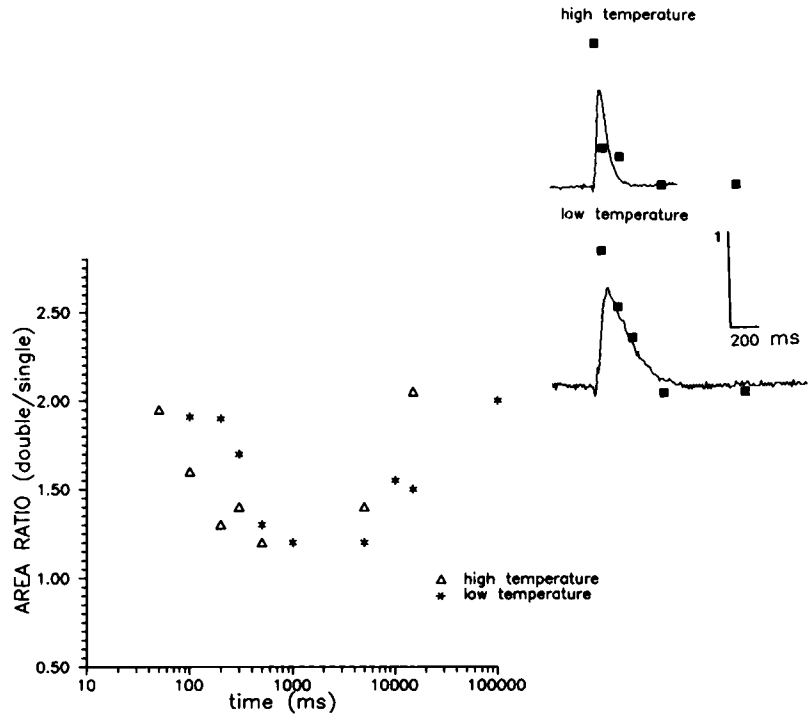

FIGURE 5 Dependence of the time-course of the desensitization of the $InsP_3$ response on temperature. Averages of desensitization ratios for 3 cells when the temperature was changed from 19–21° (high temperature) to 11–13° (low temperature) and back are shown. All cells were bathed in low Ca^{2+} medium, and the high temperature data are the same as in Fig. 3 for low Ca^{2+}. Records at high temperature were always taken before and after lowering the temperature, then averages for a given time-interval at a given temperature were calculated for each cell. Ratios were calculated as described in the caption to Fig. 3. Insets compare the normalized time-courses of the responses in one of the three cells to single injections of $InsP_3$ at high and low temperatures (*recordings*) with the time-courses of the desensitization at these two temperatures in the same cell (*rectangles*). For the normalization, the recorded time-course of the response is divided by a peak amplitude of the same response. The time-course of the desensitization is calculated as an area ratio of this cell. The initial ratio is bigger than 1 probably because of the activation of the voltage dependent conductances which results in a "facilitation" of the overlapping responses to two injections. Apart from this effect, the time-courses of the decline of the responses and the desensitization are similar at both temperatures.

comment also suggests that our measurements constitute only a lower limit on the desensitization speed; the real onset may be even faster than that observed.

We conclude that the time-course of the desensitization of the $InsP_3$ response is compatible with its playing a role in the transient nature of the $InsP_3$ response in the *Limulus* ventral photoreceptor.

Payne et al. (1990) showed that an injection of Ca^{2+} depresses the response to $InsP_3$ injected 2 s later but has no effect on the response to a second injection of Ca^{2+} delivered with the same delay. The time-course of the recovery from the desensitization is unaffected by the extracellular Ca^{2+} level (Payne et al., 1988). These observations therefore suggest that the desensitization of the $InsP_3$ response in the ventral photoreceptor may be due to the activation of a calcium-intermediated negative feedback (Payne et al., 1990) and not to the depletion of the intracellular Ca^{2+} stores (proposed by Berridge and Irvine, 1989).

Two observations suggest that the fast component of the desensitization of the $InsP_3$ response is also due to a negative feedback mechanism rather than to depletion. First, the time-course of the onset of the desensitization, like the time-course of the recovery, does not depend on the extracellular Ca^{2+}. Second, the desensitization slows the time-course of the response. This observation is compatible with the termination of the response by negative feedback but not by depletion.

Injection of $InsP_3$ causes excitation and adaptation of a local portion of the cell (Fein et al., 1984; Payne and Fein, 1987). On the other hand $InsP_3$ can diffuse throughout a cell (Fein et al., 1984). Why does $InsP_3$ not diffuse from the desensitized portion of the cell and induce Ca^{2+} release elsewhere? We suggest two possible answers. First, $InsP_3$ can be metabolized faster than it diffuses away from the desensitized zone, and second, there is a supralinear dependence of Ca^{2+} release on $InsP_3$. Such a supralinearity has been demonstrated in a number of other tissues (Parker and Miledi, 1989; Meyer et al., 1988; Meyer et al., 1989; Finch et al., 1992). In this case dilution of $InsP_3$ concentration will reduce Ca^{2+} release. The similarity between the initial

responses to the injection of $InsP_3$ and its metabolically stable analogue $InsP_3S_3$ suggests that the second possibility is more likely.

A final question is whether the time-course of the desensitization is compatible with its underlying the mechanism of light adaptation. The onset of light adaptation in the *Limulus* ventral photoreceptors was measured by superimposing test flashes of fixed energy on a step adapting stimulus (Lisman and Brown, 1975; Fein and Charlton, 1977). At low light intensities the decrease in the average response starts 200 ms after the beginning of the response to the adapting light. Grzywacz et al. (1988) measured the time-course of the change in amplitude of the individual photon responses underlying the responses to flashes of low light intensities. They observed an initial facilitation which began to decline, possibly due to the onset of adaptation, ~150 ms after the start of the response. This is therefore a lower limit on the onset of light adaptation at these (low) intensities. The onset of the desensitization as measured by us (time-constant 60–150 ms) is faster than the onset of light adaptation. This discrepancy can be explained by the spatial differences between the light stimulus and the injection stimulus. Injected $InsP_3$ is delivered to a local portion of the cell in the vicinity of the pipette and the second injection is exactly at the same site. It may take time for the desensitization induced by the individual photons of a diffuse flash to reach the sites of action of the photons from a second flash (that is, to cover the whole cell). Recovery from the adaptation is biphasic, the fast phase having a time-constant of 5–9 s and the slow one 300–500 s (Classen-Linke and Stieve, 1986). The time-course of the fast phase of the recovery is comparable with that of the recovery from the desensitization of the $InsP_3$ response.

We conclude that if $InsP_3$-induced depolarization contributes to the light response, then feedback inhibition of the response to $InsP_3$ may well underlie the light-adaptation and the fast phase of the dark adaptation.

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