Presynaptic enhancement of signal transients in photoreceptor terminals in the compound eye

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SUMMARY

Intracellular recordings show that photoreceptor axons in the first optic ganglion of the blowfly have characteristic electrical properties not seen in the soma. In the axon, a fast depolarizing transient (FDT) occurs on the rising phase of the photoreponse. The FDT is elicited by depolarizing current injections, can follow a sudden relaxation from hyperpolarization, has a distinct voltage threshold, and is accompanied by a rapid transient increase in membrane conductance. Blocking voltage-sensitive potassium conductances in photoreceptors with TEA unmasks spontaneous, long-lasting (ca. 50–150 ms) action potentials. These voltage-sensitive effects and measurements of membrane resistance suggest that the FDT is caused by a voltage-dependent increase in conductance, localized in the photoreceptor axon terminals. The transient amplifies the peak presynaptic response at least twofold, and appears to generate the fast, initial component of the transient postsynaptic response. Thus the FDT contributes to the synaptic amplification and boosting of high frequencies that enhance signals at the visual system’s first synapse.

1. INTRODUCTION

A major function of the first optic ganglion of arthropod compound eyes is signal enhancement (Laughlin 1981). Under physiological illumination conditions the photoreceptors respond to light increments and decrements with low-amplitude voltage signals, which are amplified as they pass across the array of parallel synapses connecting photoreceptors to large monopolar cells (LMCs). In addition, the photoreceptor–LMC synapse adapts, in the sense that it generates transient responses (Austrom et al. 1970; Jarvilehto & Zetler 1973; Laughlin et al. 1987). As a result the large direct current (dc) signal components produced by bright illumination are not transmitted, and the synapse can operate with a high gain, without saturating the postsynaptic response (Laughlin & Hardie 1978). The low-amplitude signals produced by objects of different reflectance are amplified relative to the background (Laughlin 1987). This system is interesting as a model of retinal processing in general, and as an example of neural processing in a set of synapses that, as in barnacle ocelli and salamander retina (Hayashi et al. 1985; Werblin 1977), adapt their transfer functions to prevailing input conditions.

The mechanisms responsible for amplifying the signal and generating transients have not yet been identified. Present evidence suggests that postsynaptic mechanisms, acting on the LMC membrane, are relatively unimportant. The transient responses do not result from desensitization of the histamine-gated chloride channels that generate the postsynaptic potentials (Hardie 1989). Furthermore, the postsynaptic LMCs are approximately Ohmic over their operating range (Laughlin & Osorio 1989; Weckström et al. 1989). Thus, with the possible exception of the L3 cells (Hardie & Weckström 1990), postsynaptic voltage-sensitive conductances have a minor role in amplifying and filtering synaptic inputs. This suggests that the LMC is an essentially passive integrator and that amplification and transience are achieved by regulating the release of the transmitter from presynaptic terminals of photoreceptors. In support of this hypothesis, the transient voltage responses of LMCs are accompanied by large transient changes of synaptic conductance (Laughlin & Osorio 1989; Weckström et al. 1989). Therefore, presynaptic processes are mainly responsible for signal enhancement. However, with the exception of barnacle ocelli (Stockbridge & Ross 1984; Stuart et al. 1986) and in contrast to vertebrates (Attwell 1986), there is no direct evidence for conductance mechanisms capable of shaping presynaptic signals in arthropod photoreceptor terminals.

We have tried to find such presynaptic enhancement by recording intracellularly from photoreceptor terminals, close to the sites of synaptic transmission. We found that terminals have unique electrical properties, not seen in the somata, and voltage-sensitive conductances produce amplified transient typical of transmission in the fly lamina.

2. METHODS

Standard techniques were used to record intracellularly from, and mark (with Lucifer Yellow), the photoreceptors and LMCs in the intact retina and lamina of adult Calliphora

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vicina (Hardie 1979; Hardie & Weckström 1990). Cell types and recording sites were established by using accepted criteria (Austrum et al. 1970; Shaw 1975; Hardie & Weckström 1990). Special attention was given to attain electrodes with suitable profiles and electrical properties, i.e. fine-tipped with relatively short shanks (see Results). We only considered high-quality photoreceptor impalements that gave input resistance of at least 30 MΩ in retina (Weckstrom et al. 1991) and 20–35 MΩ in lamina. This meant that most axon impalements were rejected. Well over 100 axon penetrations were required to produce the data presented below. Single-electrode switched clamp techniques were used to inject current and record voltage responses as previously described (Wilson & Goldner 1975; Finke & Redman 1984; Laughlin & Osorio 1989; Weckström et al. 1991). The membrane resistance changes accompanying responses were estimated by comparing responses averaged with and without the application of polarizing current (van Hateren 1986; Laughlin & Osorio 1989). In the case of extracellular TEA application, a small drop of 10–100 mM TEA was applied to a small hole cut into the dorsal cornea.

The light stimulus was either a green high-intensity light emitting diode (LED) (Stanley, U.S.A.) with peak emission at 555 nm, driven with a controlled current source, or a Xenon flash (Cathodeon Ltd, U.K.) attenuated with a set of neutral density filters. The light stimuli were calibrated in situ by counting the discreet depolarizing events (quantum bumps) produced in photoreceptors, and photometrically with a PIN-diode (in case of the LED) and with a photometer (Opti-O-Meter BIX, United Detector Technology, U.S.A.). The flies were normally allowed to dark adapt at least 1 h before recordings were made. The responses were digitized, stored and analysed by using standard on- and off-line laboratory computer techniques.

3. RESULTS

(a) A fast depolarizing transient (FDT) in the axon

The light responses recorded from photoreceptor axons in the first optic neuropile, the lamina, clearly differ from those recorded from photoreceptor somata in the retina (figure 1). In the axon a prominent FDT is superimposed on the rising phase of the response. It must be stressed that we only record the FDT with fine-tipped electrodes with resistances in excess of 150 MΩ (see Methods). These electrodes achieve reasonable quality impalements, as indicated by axon input resistances of 20 MΩ or more. Blunter electrodes, with resistances less than 100 MΩ, fail to record the FDT and register axonal input resistances that are usually less than 10 MΩ. The blunter electrodes probably damage the axonal membrane and induce a leakage conductance that inactivates the FDT by depolarizing the membrane or short-circuiting the other axonal conductances. With sharp, high-quality electrodes inserted in photoreceptor somata we also observe a small deflection, or notch, in the rising phase when recorded in proximal retina (figure 1a; see also Shaw (1984)). Taken together these observations suggest that the FDT is a physiological voltage response, generated in relatively undamaged photoreceptor axon, and only transmitted with considerable decrement backwards to the soma.

Figure 1. Intracellular recordings of the responses to brief light flashes show that a fast depolarizing transient appears in the axon (a) against (b) and is transmitted to an LMC (c). Recordings from (a) the photoreceptor soma in the proximal retina with resting potential of −62 mV, (b) the photoreceptor axon terminal in the lamina, with resting potential −59 mV, and (c) the postsynaptic monopolar interneuron (LMC), resting potential −38 mV. The 10 μs light flash (having relative intensity of 100, 49, 14, 2.5 and 0.09, delivering maximally about 5000 effective photons) was presented at the beginning of each trace to a stably dark-adapted retina. All responses are from different specimens, and the effective intensities are not necessarily equivalent.
a large hyperpolarization (40 mV below rest) but one only needs to hold the cell at a slightly more depolarized level to restore the transient. In addition, in single-electrode clamp experiments the amplitude of transients does not increase linearly with injected current. Whereas the steady-state plateau response to current exhibits the rectification that is characteristic of fly photoreceptor somata (Weckström et al. 1991), the amplitude of the initial transient response shows an opposite accelerating nonlinearity (figure 3c). These observations suggest that voltage-sensitive conductances generate the FDT. This hypothesis is supported by the fact that the FDT is readily elicited by current injection alone, either upon depolarization or following the release from hyperpolarization (figure 2).

Can we measure conductance changes underlying the FDT? The small diameter of axons ( < 2.0 μm) and the relative inaccessibility of the lamina necessitate single-electrode techniques. The absolute requirement for high-resistance pipettes prevents us from voltage clamping the terminals. We can, however, measure the changes in membrane resistance accompanying the FDT, by comparing the responses elicited with and without the injection of small polarizing currents (van Hateren 1986; Laughlin & Osorio 1989). The apparent input resistance of the photoreceptor axon terminals drops by as much as 5 MΩ during the FDT and this far exceeds the changes in apparent resistance associated with the remainder of the depolarizing light response (figure 3a). As one might expect from the large decrement in FDT amplitude, this fast conductance change is not recorded in photoreceptor somata, supporting the hypothesis that the FDT is a local phenomenon confined to the axon. During the
response to a large-contrast stimulus the drop in input resistance of the photoreceptor soma can be much larger, from a dark input resistance of about 30 MΩ to only a few megaohms (Weckström et al. 1991). This is to be expected on the basis of the photoreceptor geometry and because the light-gated conductance introduces a large shunt in the soma.

These experiments suggest that the membrane of photoreceptor axon terminals contains a unique set of voltage-sensitive conductances capable of producing small regenerative transients. Presynaptic calcium conductances are obvious candidates, and the blockage of potassium conductances is known to unmask voltage-sensitive calcium conductances (for a review see Hille (1992)). We explored this possibility by applying TEA in high concentration to the retinal extracellular space (see methods). This treatment blocks a prominent delayed rectifier in the photoreceptor membrane (Laughlin & Weckström 1989; Weckström et al. 1991), and we found that it increases the membrane resistance of the somata from 30 MΩ to 40–50 MΩ. With extracellular TEA, large spontaneous depolarizing transient events are generated in photoreceptors. They last 100–150 ms and cause transmitter release, as demonstrated by recordings of spontaneous hyperpolarizing events from postsynaptic LMCs (figure 4). The duration of these spontaneous depolarizations is quite variable depending, among other things, on the effective TEA concentration in retinal extracellular space. The timecourse of pre- and postsynaptic spontaneous events is very much the same when recorded in the same preparation. The frequency of these spontaneous events also depends upon the resting membrane potential. Applying TEA depolarizes photoreceptors by 10–20 mV, from a normal resting potential of around −65 mV. Hyperpolarizing the TEA-treated photoreceptors by more than 30 mV blocked these spontaneous events, and the spiking rhythm could be reset by changing membrane potential, either by illuminating the cell or by injecting current. These observations support the hypothesis that fly photoreceptors—very probably their axons—contain voltage-sensitive conductances capable of generating

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**Figure 4.** Spontaneous spikes recorded in the somata of photoreceptors that have been poisoned by prolonged exposure to high extracellular concentrations of TEA. (a) A typical spike recorded from a photoreceptor. (b) A similarly spontaneous hyperpolarizing event appearing in first-order interneurons, the LMCs postsynaptic to the photoreceptors. (c) When recorded successively from the same preparation and normalized to the same amplitude and polarity the photoreceptor and LMC spontaneous spikes have almost identical waveforms, suggesting that the signal is faithfully transmitted across the synapse under these conditions.

**Figure 5.** The influence of the FTP on the coding of light intensity. (a) Voltage responses recorded from a terminal when dark adapted elicited with a small number of photons. LED flashes of 1 ms duration with intensity increasing from about 10 effective photons per flash to about 400 effective photons per flash. (b) Comparison of the average of peak response amplitudes obtained in three recordings from dark-adapted photoreceptors in the retina (squares) and in the lamina terminals (stars) in the function of the stimulus intensity as effective photons per flash. Note that the FTP consistently amplifies the peak response. (c) A pair of axon terminal responses to an increment and a decrement of intensity of low contrast, recorded in the light-adapted state. The FTP is present at both the onset of an increment and the offset of a decrement, in the physiological response range where LMC responses are unsaturated. Stimulus contrast = 0.11, background = about 7 × 10⁹ effective photons s⁻¹.
transient regenerative depolarizations (Barash et al. 1988; Rubinstein et al. 1989).

(c) Does the FDT influence signal transmission?

We compared the amplitudes and waveforms recorded from photoreceptor somata, photoreceptor terminals and LMCS. These responses were recorded individually, at different times. Consequently, to prevent artefacts, great care was taken to compare responses recorded under conditions when cells received stimuli of equal brightness. In dark-adapted photoreceptors, the FDT is elicited by flashes of low intensity, and this both increases the peak amplitude of the responses (figure 5) and advances the time-to-peak. Thus both the absolute amplitude of the response and the rate of change of amplitude are increased. Furthermore, the comparison of axonal and LMCS responses suggests that the FDT is transmitted across the synapse (figure 1) to produce the fast initial transient hyperpolarization.

Synaptic transmission from photoreceptors to LMCS selectively amplifies high frequencies (Järvelihto & Zettler 1971; French & Järvelihto 1978; Laughlin et al. 1987). The FDT is capable of boosting high frequencies, and this is illustrated by comparing the frequency responses of the soma and terminal, estimated by taking the Fourier transform of the light-initiated impulse response (figure 6). The FDT causes a clear hump, or increase in gain, in the frequency range 100–300 Hz, which is missing in the soma recordings. This is the range in which synaptic transmission adds noise (Laughlin et al. 1987) and in which the synapse most strongly amplifies (French & Järvelihto 1978). It is also the frequency range associated with the oscillations that are sometimes recorded from LMCS (van Hateren 1987). Note that, on occasions, similar oscillations are evoked in axon terminal by injected current (figure 3b).

4. DISCUSSION

(a) Limitations to the analysis of the FDT

We report a new phenomenon in fly photoreceptors, a fast depolarizing transient recorded from the axon terminals. The recordings necessary to observe this phenomenon are technically demanding, and this may explain why it has not been reported before in the fly (but see Shaw (1979, 1984)). The small axon diameter limits both the duration and the frequency of successful intracellular recordings. These problems also impeded the detailed comparison of soma, axon and LMCS responses needed to define accurately the contribution of the axonal membrane to signal enhancement. In addition, the absolute requirement for high-resistance micropipettes prevented us from analysing axonal conductances in situ by voltage clamping the terminals. These difficulties were compounded by a second problem. For most of their length, the photoreceptor axons lie within lamina cartridges. These local compartments restrict free diffusion in the lamina, impeding ionic substitution and the application of pharmacological agents. None the less, the evidence we discuss clearly suggests a new aspect of signal processing in the insect lamina, namely the enhancement of fast signals by voltage-sensitive mechanisms in the presynaptic terminals of photoreceptors.

(b) Signal enhancement in photoreceptor synaptic terminals

We have shown that the FDT represents a set of mechanisms which can amplify the graded voltage signals generated by phototransduction. This suggests a design principle, shared with vertebrate photoreceptors, namely the use of voltage-sensitive mechanisms, localized to a discrete proximal region of the cell, to filter an shape signals before transmission (Attwell 1986). The FDT is evidently responsible for the fast rapid transient at the onset of the LMCS response (figure 1). Our best recordings suggest that the FDT can amplify the peak on-transient amplitude by a factor of 1.5 to 2.0 (figure 5). Thus the FDT can contribute substantially to an overall synaptic gain of 6 (Laughlin et al. 1987). In addition, the FDT boosts signals in the band 100–300 Hz, where the synapse has the highest gain (Järvelihto & Zettler 1971; French & Järvelihto 1978; Laughlin et al. 1987; Järvelihto et al. 1989).

Our observations cannot account for all the transformations that take place during synaptic transmission.
from photoreceptor to LMC. These include the elimination of a sustained response to constant light via a slower decay of postsynaptic response, lateral inhibition, and the production of pronounced off-transients, particularly in light-adapted cells (Zettler & Järviheilo 1972; Laughlin & Hardie 1978). Although present evidence suggests that many of the mechanisms responsible for these components are acting on the photoreceptor terminals (Wang-Bennett & Glantz 1987; Laughlin & Osorio 1989; Weckström et al. 1989), our recordings of presynaptic voltage responses have failed to detect reliable electrical correlates of these processes.

(c) Mechanisms underlying the FDT

Our data suggest that the FDT is generated by voltage-sensitive conductances localized in the axon terminals. The large transient increase in conductance that accompanies the FDT may indicate the successive activation of inward and outward currents, which could plausibly be responsible for the initial fast depolarization and its repolarization, respectively. Voltage-sensitive potassium conductances, capable of generating large rapid outward currents, are found on the somata of fly photoreceptors (Laughlin & Weckström 1989; Weckström et al. 1991; Hardie 1991) and could, therefore, also be active in the membranes of the axon terminals. The conductances generating the inward current appear to be voltage dependent. Each photoreceptor terminals makes approximately 200 tetradic synapses with second-order cells (Nicol & Meinerzhagen 1982), so a voltage-sensitive Ca²⁺ conductance associated with synaptic transmission is a reasonable candidate. Calcium spikes are often unmasked by potassium channel blockers (Hille 1992), and this could explain the regenerative events recorded intracellularly following the application of TEA (figure 4). It has also been suggested that the extracellular spikes observed during the early stages of retinal degeneration in Drosophila are calcium spikes (Rubinstein et al. 1989). Prominent calcium conductances are localized at graded potential synapses made by barnacle photoreceptors (Stockbridge & Ross 1984), and calcium-sensitive conductances are thought to accelerate the rising phase of the synaptic response in the crab sensorimotor system by positive feedback. This boosts high frequencies to achieve capacity compensation (Bligh & Linas 1980).

Voltage-sensitive sodium channels could also contribute to the FDT. TTX-sensitive action potentials were recorded from photoreceptor cell bodies in drone bees, and fast depolarizing transients similar to those reported here in fly axons were recorded from the cell bodies of worker bee photoreceptors. In drones, the TTX-sensitive conductance both amplifies and accelerates small voltage responses and enhances the signals generated by small, rapidly moving targets, such as queen bees (Coles & Schneider-Picard 1989). This type of enhancement mechanism may be widely used in the eyes of fast-flying diurnal insects, but is usually confined to the area where signal enhancement is most effective, the lamina terminals.

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