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RAPID PUBLICATION

Tonic Transmitter Release in a Graded Potential Synapse

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SUMMARY AND CONCLUSIONS

1. We studied graded synaptic transmission in the fly photoreceptor-interneuron synapse by using intracellular in situ recordings from pre- and postsynaptic cells.

2. A large presynaptic hyperpolarization after light adaptation, caused by the activation of the electrogenic Na^+/K^+ pump, drastically reduced the conspicuous postsynaptic dark noise. At the same time, the postsynaptic neurons depolarized, with an increase of input resistance of 5–10 MΩ.

3. The spectral characteristics of the postsynaptic membrane noise in dark and during noise reduction, together with the other results, suggested that the transmitter release decreased dramatically ~12 mV below the resting potential of the presynaptic photoreceptors.

4. During the postsynaptic noise reduction, the saturated and subsaturated first-order visual interneuron responses were increased up to 9 mV with a time constant of recovery of ~10 s. This increase was shown to be caused by the negative shift of the reversal potential of the transmitter-gated (mainly Cl^-) conductance, caused apparently by the reduced transmitter input.

5. The results strongly suggest that the photoreceptor transmitter release in fly is tonic, even in dark, and further support the modulation of the synaptic voltage transfer by postsynaptic Cl^- extrusion.

INTRODUCTION

In the blowfly (*Calliphora vicina*) compound eye, the columnar first-order visual interneurons (LMCs) receive multiple synaptic input from six photoreceptors (Kirschfeld 1967). The light-induced graded depolarization in photoreceptors drives the release of the photoreceptor transmitter histamine (Hardie 1987), which binds to postsynaptic fast Cl^- channels, causing a graded hyperpolarization (Hardie 1989; Zettler and Straka 1987). The general properties of this synapse are well known (Autrum at al. 1970; Laughlin 1987). The transmitter has previously been proposed to be released tonically, even in darkness (Laughlin et al. 1987), causing a considerable Cl^- load on the postsynaptic neurons, requiring an efficient Cl^- extrusion mechanism (Uusitalo and Weckström 1994). In this study we show that the transmitter release in photoreceptor-LMC synapse is indeed tonic, and we report that the postsynaptic responses are enhanced after a block of this tonic release.

METHODS

Intracellular in situ recordings from presynaptic photoreceptors and postsynaptic LMCs in the compound eye of the blowfly *C. vicina* were conducted with conventional methods (Laughlin and Hardie 1978). The fly was grounded from the head capsule with a silver–silver chloride wire, and the cells were approached via a hole made in the dorsal margin of the cornea. The glass capillary microelectrodes (Clark Electromedical, UK), pulled with P-80 PC (Sutter Instruments), were filled with 2 M potassium acetate with 5 mM KCl (tip resistance 100–150 MΩ) and moved with a piezoelectric stepper (PZ-550, Burleigh). A Xenon lamp (Hamamatsu, Japan) with a shutter (Unibliz 132, Germany) was used to give the adapting light. The test flashes were applied with a Xenon flash unit (Cathodeon, UK)

The power spectra of the intracellularly recorded voltage samples were calculated via fast Fourier transform using standard methods (Bendat and Piersol 1971; Juusola et al. 1994) using a Blackman-Harris four-term window (Harris 1978). Current-clamp experiments were performed according to standard methods (Finkel and Redman 1984; Weckström et al. 1991) after critical capacitance compensation. The switching frequency was 3 kHz and the time constant of the electrode (after capacitance compensation) was ~5 μs.

To be qualified as results the cells recorded had to fulfill a tight set of electrophysiological criteria. High-quality photoreceptor recordings were characterized by resting potentials (RP) of −60 mV, high input resistance (IR) of 30 MΩ, and large dark-adapted peak light-on responses (ORs) >50 mV (Weckström et al. 1991). The LMC recordings were accepted when RP was from −40 to −60 mV, IR was from 20 to 40 MΩ, and OR was greater than −35 mV (Hardie and Weckström 1990).

RESULTS

The results in this paper are based on recordings from 20 LMCs and 20 photoreceptors. First we studied the changes in the pre- and postsynaptic RP after light adaptation. Intense light stimulation induced a large hyperpolarizing afterpotential in photoreceptors (Fig. 1*A*), which was due to the increased activity of the electrogenic Na^+/K^+ pump, as reported earlier (Hamdorf et al. 1988; Jansonius 1990). Intracellular recordings from presynaptic photoreceptors showed clear dependence of this afterpotential on the duration of the adapting light stimulus (Fig. 2*C*). During the presynaptic pump potential the postsynaptic neurons depolarized by 2–6 mV and the conspicuous postsynaptic noise was drastically reduced (Figs. 1*A* and 2*B*). In this same period the IR of the LMCs increased by 7.4 ± 2.9 (SD) MΩ ($n = 5$). These findings suggest that the tonic (dark) transmitter release from the photoreceptors was strongly reduced during the presynaptic pump potential. The power spectrum of the dark noise in postsynaptic LMCs normally consists of two clearly distinguishable components (Laughlin et al. 1987). The low-frequency component of the spectrum was attenuated considerably during the presynaptic pump potential, i.e., during the noiseless and depolarized period of the postsynaptic LMCs (Fig. 1*B*). This further supports the hypothesis of the tonic transmitter release.

The conspicuous postsynaptic noise reappeared at the time when the presynaptic hyperpolarization recovered to the

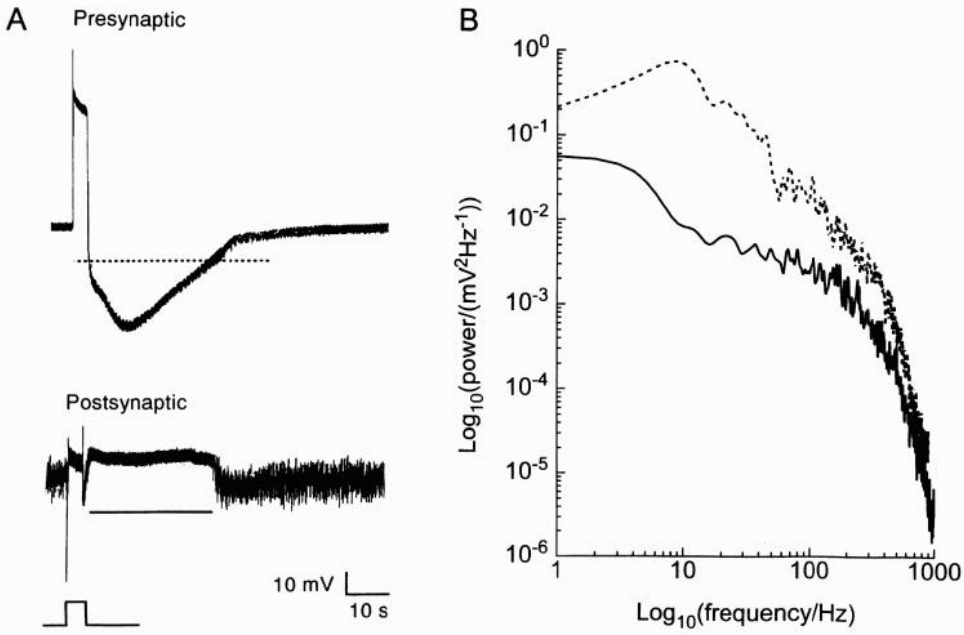

FIG. 1. *A*: response of the presynaptic photoreceptor (*top*) and the postsynaptic 1st-order visual interneuron (LMC) (*bottom*) to a 2-s adapting stimulus (10^7 effective photons per s, denoted by the pulse below). Note the virtually noiseless, depolarized (2–6 mV) period of the postsynaptic LMC (——) coinciding with the presynaptic pump potential (as in Fig. 2, *A* and *B*). Dotted line in *top panel*: voltage below which the transmitter release decreased. *B*: power spectrum of the LMC voltage noise before the adapting stimulus (- - -) and during the virtually noiseless and depolarized period of the LMCs (——). Note the disappearance of the low-frequency component indicating reduction of the tonic transmitter release (Laughlin et al. 1987) during the period of presynaptic pump potential.

voltage of 12 ± 1 mV (n = 15, both for photoreceptors and LMCs) from the RP (Fig. 1*A*). This implies that the transmitter release has a strong dependence on the presynaptic membrane potential such that the transmitter release is much reduced below about −73 mV, when normally the dark RP in photoreceptors was −61 ± 7 mV (n = 20).

The properties of synaptic voltage transfer were studied by applying test flashes of varying intensity before and during the presynaptic pump potential. Under this period the postsynaptic responses increased in amplitude up to 9 ± 0.2 mV (n = 15). This increase was largest with a 2-s adapting light stimulus and with test flashes that saturated the postsynaptic LMC voltage (Fig. 2). The period of the increased response amplitude and the period of the reduced noise in postsynaptic LMCs both closely matched the period when the presynaptic pump potential was 12 mV more negative than the RP (Fig. 2). The increase of the amplitude of the LMC responses depended directly on the presynaptic pump potential (Fig. 2*C*). With adapting pulses >2 s, the depolarizing afterpotential (PDA and/or non-PDA) in presynaptic

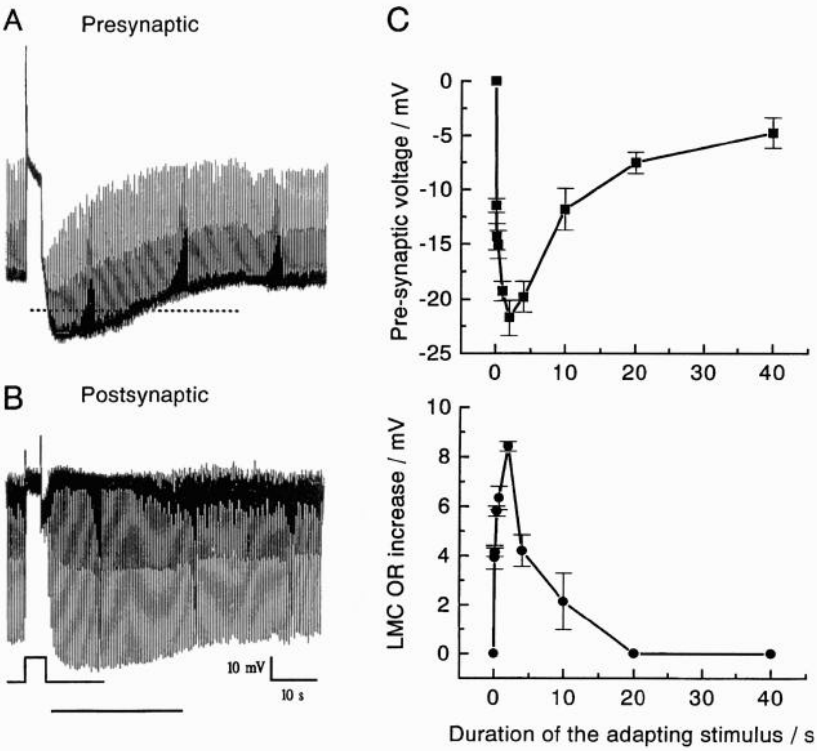

FIG. 2. *A*: responses of the presynaptic photoreceptor to continuous stimulation with 2-ms flashes. The 2-s adapting stimulus (the pulse below Fig. 1*B*, 10^7 effective photons per s) depolarizes the photoreceptor and induces an afterhyperpolarization of ~20 mV that is caused by the activation of the electrogenic Na^+/K^+ pump (Hamdorf et al. 1988; Jansonius 1990). Dotted line: voltage below which the transmitter release is drastically reduced (see Fig. 1). *B*: responses of the postsynaptic LMC under identical stimulation conditions as in *A*. The test flash causes saturating (hyperpolarizing) responses in the LMC. Note the ~10-mV increase in the response amplitude in the postsynaptic LMC (——). *C*: dependence of the presynaptic pump potential (*top*) and postsynaptic increase in the light-on response (OR) amplitude (*bottom*) on the duration of the adapting stimulus delivered as described in Fig. 2*A* (symbols denote mean ± SD, n = 5). The voltage of the hyperpolarizing pump potential corresponds to the amplitude of the increased ORs. At *top*, 0 mV corresponds to the resting potential of −61 ± 7 mV (n = 20). At *bottom*, 0 mV corresponds to the amplitude of the control ORs in the LMCs.

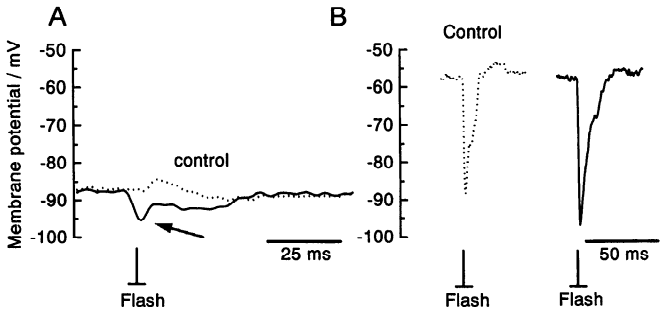

FIG. 3. *A*: responses of the postsynaptic LMC to a 2-ms flash during a steady-state current (−2.1 nA) delivered through the recording microelectrode. The figure shows where the postsynaptic response was just reversing before the period of increased ORs, at −88 mV (control, · · ·). *Bottom trace*: increased OR during the period of presynaptic pump potential (\, ——). Note that the negative shift in the equilibrium potential of the synaptic current (E_{rev}) during this period is clear. This shift was ≥9 mV in the shown recording and thus can explain the increase of the ORs during the presynaptic pump potential, which was 9 ± 0.2 mV (n = 15), see Fig. 2. *B*: OR of the LMCs to the same 2-ms flash without the negative current. During the presynaptic pump potential the LMC responses are clearly enhanced (*right,* ——) compared with normal ORs (*left,* · · ·).

photoreceptors (Horridge and Tsukahara 1978; Minke and Kirschfeld 1984) effectively antagonizes the Na^+/K^+-pump-induced hyperpolarization and thus decreases the postsynaptic reponse. With subsaturating test stimuli, the LMC response were still increased after the adapting light pulse. However, with very small-intensity stimulations (<100 photons per flash) the LMC responses were smaller than normal. This is understandable, because the transmitter release is likely to be almost completely stopped with the large presynaptic hyperpolarization and the small presynaptic depolarizations do not cause much transmitter release.

The increased LMC response may be directly explained on the basis of the continuous transmitter release. Both the postsynaptic membrane potential and the reversal potential for the transmitter-gated current have been shown to be affected by an electrogenic Cl^- transport, which is crucial for the maintenance of Cl^- homeostasis in these small neurons (Uusitalo and Weckström 1994). We studied the possible shifts in the reversal potential of the transmitter-gated current with current pulses delivered into the postsynaptic LMCs during the voltage response to saturating test flashes. The LMC reversal potential was indeed hyperpolarized during the presynaptic pump potential (Fig. 3). The actual change of the equilibrium pontential of the synaptic current (E_{rev}) cannot be accurately established for technical reasons, because the perfect current clamping of the OR during the limited time the increased response lasted was not possible. The negative shift of the E_{rev} was ≥9 ± 3 mV (n = 5) because the reversal potential of the OR was hyperpolarized by this value from the E_{rev}, determined by current clamp before the presynaptic hyperpolarization (as in Fig. 3). This indicates a causal relationship to the increase in LMC response amplitude, which was 9 ± 0.2 mV (n = 15).

DISCUSSION

According to the present results, the transmitter release in the fly photoreceptor-interneuron synapse appears to be continuous even in dark. In this sense the synapse is similar to those formed by vertebrate photoreceptors (Baylor and Fettiplace 1976; Dowling and Ripps 1973). There seems to be a relative threshold for the transmitter release, because the LMC responses can only be observed above a presynaptic membrane potential of ~12 mV less negative than the RP. Below this level the transmitter release indeed diminishes rapidly, as evidenced by the drastic noise reduction in the postsynaptic LMC (Fig. 1). During this noise reduction period the LMC responses are even larger, especially with stimuli that saturate the hyperpolarizing response. The enhancement is caused by a transient hyperpolarizing shift in the E_{rev} (Fig. 3). This is most probably brought about by the action of the tonic Cl^- extrusion mechanism (Uusitalo and Weckström 1994) in the absence of a tonic-transmitter-mediated Cl^- influx, causing a reduction in intracellular $[Cl^-]$. Although the increase of the postsynaptic reponse amplitude is caused by relatively unphysiological stimulation, it is an indicator of the underlying synaptic adaptation mechanisms. The manipulation of the synaptic transmitter release in the manner described in the present work could be an important tool in further investigations of this graded synaptic transmission. The shifts in Cl^- reversal potential described in this work and previously (Uusitalo and Weckström 1994) suggest a possible way for the gain regulation in the synapse.

The continuous transmitter release in the photoreceptor-LMC synapse is likely to be an adaptation to increase gain and sensitivity of the visual system. Having a tonic, as opposed to pulsed, transmitter release mechanism allows the photoreceptors to transmit signals of both polarity, i.e., reponses to both positive and negative contrasts with high gain (Howard et al. 1987; Juusola 1993; Juusola et al. 1994, 1995; Laughlin et al. 1987). The dipteran photoreceptor-LMC synapse has been called a high-gain synapse (Laughlin et al. 1987). This property is partially invested in the properties of the postsynaptic histamine-gated Cl^- channels, which appear to have a high cooperativity (Hardie 1989) and, accordingly, a steep dependence of their open probability from the transmitter concentration. However, the continuous transmitter release is equally important as a mechanism that forces the synaptic voltage transfer to operate near the steepest part of the governing function, regardless of the presynaptic DC voltage (Laughlin et al. 1987), which is in turn largely defined by the adapting intensity of the ambient light.

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