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MEMBRANE CONDUCTANCES INVOLVED IN AMPLIFICATION OF SMALL SIGNALS BY SODIUM CHANNELS IN PHOTORECEPTORS OF DRONE HONEY BEE

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SUMMARY

1. Voltage signals of about 1 mV evoked in photoreceptors of the drone honey bee by shallow modulation of a background illumination of an intensity useful for behaviour are thought to be amplified by voltage-dependent Na^+ channels. To elucidate the roles of the various membrane conductances in this amplification we have studied the effects of the Na^+ channel blocker tetrodotoxin (TTX) and various putative K^+ channel blockers on the membrane potential, V_m.

2. Superfusion of a slice of retina with 0·5–10 mM-4-aminopyridine (4-AP) depolarized the membrane and, in fifty of sixty-three cells induced repetitive action potentials. Ionophoretic injection of tetraethylammonium produced similar effects.

3. In order to measure the depolarization caused by 4-AP, action potentials were prevented by application of TTX: 4-AP was applied when the membrane was depolarized to different levels by light. 4-AP induced an additional depolarization at all membrane potentials tested (-64 to -27 mV). We conclude that there are 4-AP-sensitive K^+ channels that are open at constant voltage over this range.

4. 4-AP slowed down the recovery phase of the action potential induced by a light flash by a factor that ranged from 0·51 to 0·16. This reduction could be accounted for by the reduction in a voltage-independent K^+ conductance estimated from the steady-state depolarization.

5. After the voltage-gated Na^+ channels had been blocked by TTX, exposure to 4-AP further changed the amplitude of the response to a small ($\approx 10\%$) decremental light stimulus. The change was an increase when the background illumination brought V_m to potentials more negative than about -40 mV; it was a decrease when $V_m > -40$ mV. The data could be fitted by a circuit representation of the membrane with a light-activated conductance and a K^+ conductance ($E_K = -66$ mV) that was partly blocked by 4-AP. The voltage range studied was from -52 to -27 mV; neither conductance in the model was voltage dependent.

6. The responses to small changes in light intensity in the absence of TTX were mimicked by a model. We conclude that a voltage-dependent Na^+ conductance described by the Hodgkin–Huxley equations can amplify small voltage changes in a

cell membrane that is also capable of generating action potentials; the magnitude of the K^+ conductance is critical for optimization of signals while avoiding membrane instability.

INTRODUCTION

This work is an attempt to describe how membrane conductances are combined in a neuron whose Na^+ channels function not to produce action potentials but to amplify and speed up the small voltage changes that result from a physiological input. The neuron studied is the large photoreceptor cell of the compound eye of the honey bee drone.

The drone photoreceptor is like that of most other invertebrates in that it depolarizes in response to light, but somewhat unusual in that it appears to have evolved to be effective for one particularly well-defined task, the detection of dark spots in the sky that might be queen bees (Coles & Vallet, 1991; see Kirschfeld & Wenk, 1976, for another species). Drones respond behaviourally to an object that produces a 6% decrease in intensity of the light incident on one ommatidium (Vallet & Coles, 1991); a similar stimulus in the laboratory produces a hyperpolarization of the photoreceptor membrane of less than 1 mV (Coles & Schneider-Picard, 1989*a*). It is unlikely that action potentials are used to transmit this information to the axon terminals, and certainly there is no obvious necessity. The axons are short (about 200 μm), and electrotonic propagation of graded potentials would be possible and would transfer more information than would action potentials (see Shaw, 1981; Coles & Vallet, 1991). In other insect species, intracellular recordings from the photoreceptor synapses show that graded potentials are transmitted to the second-order cells (Laughlin & Hardie, 1978; Shaw, 1981). Despite this, drone photoreceptors do have voltage-gated Na^+ channels that are capable of generating action potentials although, in almost all laboratory preparations, only in response to an abrupt increase in light intensity by a factor of more than about two (Baumann, 1968; Fig. 4*B* below). Less marked regenerative behaviour is seen in several other types of cells that are thought not to produce action potentials, including *Limulus* ventral photoreceptors (Millechia & Mauro, 1969), the T fibre of the thoracic coxal muscle receptor organ of *Carcinus* (Bush, 1981), smooth muscles cells of the rabbit pulmonary artery (Okabe, Kitamura & Kuriyama, 1988) and, under certain conditions, vertebrate rods (Fain, Quandt & Gerschenfeld, 1977).

Coles & Schneider-Picard (1989*a*) found that responses of drone photoreceptors to 30 ms decrements, or increments, of 8·9% of a background light intensity were reduced in amplitude after application of the Na^+ channel blocker tetrodotoxin (TTX). At an optimal background intensity, that depolarized the cell membrane to -38 mV, TTX decreased the peak-to-peak response by a factor of 0·4. They concluded that voltage-gated Na^+ channels were amplifying the small signals. The essence of the hypothesis is simple: at -38 mV a small fraction of the Na^+ channels are open, a small hyperpolarization closes some of these and thereby makes the membrane more polarized, amplifying the response. Aspects that were not obvious to us included the following. (1) Will this mechanism give an amplification as great (more than twofold) as that observed? (2) Will the amplifier be stable? (3) What membrane conductances are present to give the observed time course, which usually

includes an overshoot at 'on' and 'off' (Fig. 8*A*)? (4) Can a single class of voltage-dependent Na^+ channels produce both the relatively rapid full-blown action potential and also the much slower damped oscillations often associated with the amplification?

The contribution of Na^+ and K^+ conductances to small perturbations of membrane potential (V_m) have been analysed, experimentally and theoretically, in squid axon, by Hodgkin & Huxley (1952) and by Mauro, Conti, Dodge & Schor (1970) but only for the case of perturbations from the resting potential, and this in a cell whose function is to produce action potentials. Membrane potential oscillations in hair cells, which contribute to their frequency selectivity, have been described in terms of Ca^{2+} and Ca^{2+}-activated K^+ conductances (Ashmore & Attwell, 1985; Hudspeth & Lewis, 1988). Much work has been done on the generation of repetitive action potentials (reviewed in Jack, Noble & Tsien, 1983). The amplification in drone photoreceptors is different from any of these in that it involves small potentials in the range where the curves for steady-state inactivation and activation of the voltage-dependent Na^+ conductance overlap, and this conductance plays a major role. As we shall show, a voltage-dependent K^+ conductance is neither necessary nor strongly present.

Drone photoreceptors are not suited to voltage clamping: the cells are elongated, electrically coupled and receive unidentified substances from their glial cells (Shaw, 1969; Tsacopoulos, Coles & Van de Werve, 1987). We have therefore looked at effects on V_m of putative selective blockers of membrane conductances, particularly K^+ conductances, about which almost nothing was known. By using a range of light stimuli, including small incremental and decremental stimuli produced by a stimulator more flexible than that used by Coles & Schneider-Picard (1989*a*), we obtained new information about the membrane conductances. We then used this, together with existing information, in calculating responses like the observed ones from mathematical descriptions of the supposed underlying conductances, paying particular attention to the amplification. Some preliminary results have been published in abstracts (Coles, Eilbeck, Scott & Zeumer, 1991; Vallet, Coles & Eilbeck, 1991).

METHODS

Preparation. Slices of drone retina were prepared and superfused basically as described previously (Coles & Orkand, 1983; Coles & Schneider-Picard, 1989*a*). Drones were obtained from Mr N. Merin, Shikun Amal, Hadera 38244, Israel, or Mme A. Dittlo, Villandraut, France. With the isolated head held down with wax melting at 37 °C (Eicosan, Fluka, Switzerland) a slice of the head was cut with a razor blade in which the retinal parts were 400–800 μm thick, the surfaces being parallel to the long axes of the photoreceptors. The slice was held down on the floor of a chamber with one retina over a channel in the floor 1·7 mm wide so that it was superfused on both faces. The basic Ringer solution contained (mM): NaCl, 270; KCl, 7·5; $MgCl_2$, 10; $CaCl_2$, 1·6; NaMOPS (3-(*N*-morpholino)propane-sulphonic acid, hemisodium salt, Sigma), 10; and it was saturated with O_2. The pH was 7·2, temperature 20–23 °C, and flow rate about 0·2 ml s^{-1}. This superfusate differs from that of Coles & Schneider-Picard (1989*a*) in containing more NaCl, and using MOPS instead of HCO_3–CO_2 as buffer.

Light stimulation. The light source was a 50 W halogen lamp powered by a stabilized DC supply. An image of the condenser aperture was focused on the end of a 75 cm fibre light guide; a converging lens on its other end was positioned 2–3 cm from the retinal slice. The light could be turned on and off by an electromechanical shutter (Vincent Associates, USA) and steady intensities were adjusted by use of neutral density filters (Balzers, Liechtenstein). A liquid crystal pi-cell

(127S-171, EEV, UK) was used to modulate the light intensity rapidly. Command signals were generated by a Macintosh II computer with a Maclab interface using the program Scope (W.P.I., USA); these signals modulated a 1·67 kHz square wave driving voltage (Fig. 1). Deep modulation caused colour changes, so when the pi-cell was used, an interference filter with

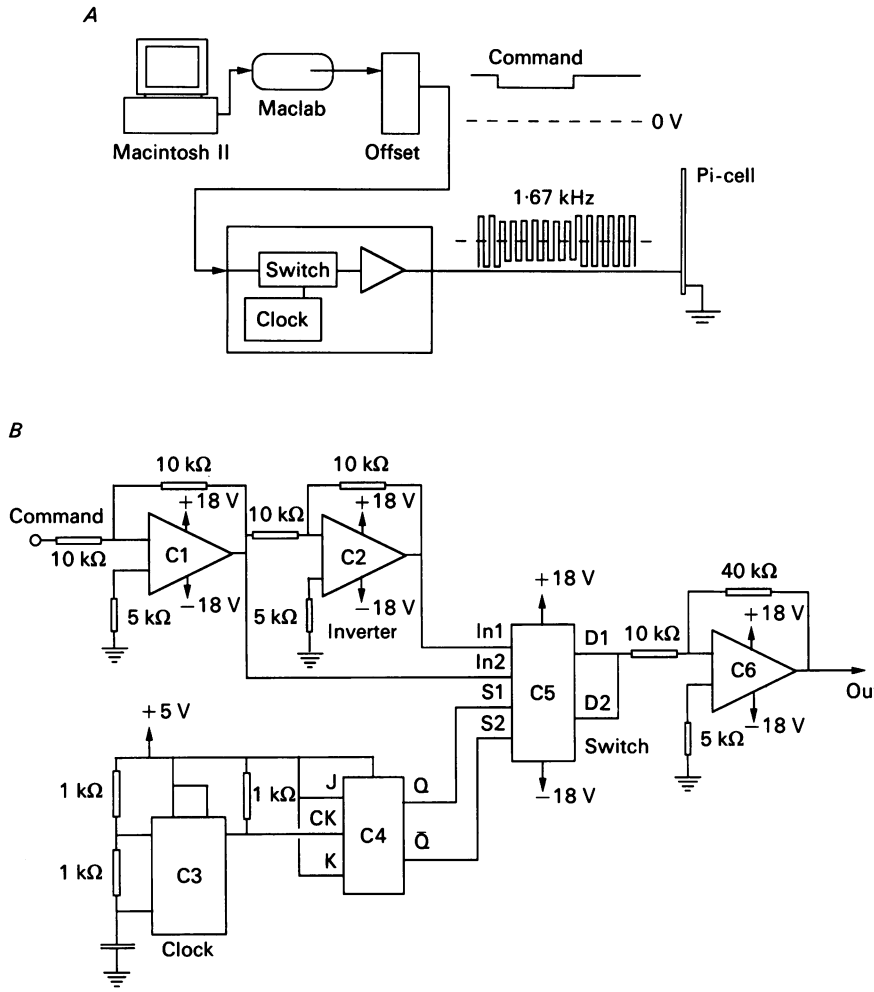

Fig. 1. *A*, arrangement used for modulating light intensity. *B*, the circuit of the switch. The components are: C1, C2, C6, UA741; C3, NE555; C4, 74LS76; C5, DL200. The circuit was designed and built by Mr J. Plantard.

maximum transmission at 450 nm and half-maximal transmission at ±2·5 nm was placed in series. The stimuli were measured as fractional changes in intensity with a photodiode (OSD 100-1, Centronic, UK). The unattenuated intensity at the surface of the retina was about 10^{16} photons mm^{-2} s^{-1} (the diode counted photons) with white light and $2{\cdot}5 \times 10^{12}$ photons mm^{-2} s^{-1} with the 450 nm filter. When the 450 nm filter and the pi-cell were placed in the light beam it was necessary to reduce the attenuation by 1·8 log units to restore the response of a photoreceptor to its amplitude in white light.

Recording arrangements. Microelectrodes normally contained 1 M-KCl, had a resistance of 70–350 MΩ and were connected to an Axoclamp-2A amplifier (Axon Instruments, USA). Voltages

were recorded continuously on a chart recorder, and responses to stimuli were filtered with a time constant of 0·5 ms, sampled at 40 kHz with the Maclab interface using the Scope program and stored on a hard disc. With small decremental stimuli, a number of responses, usually 50 or 100, were averaged. Recordings were accepted from cells with stable resting potentials in the dark more negative than −50 mV.

In some experiments root mean variance of the voltage was measured with a circuit based on an LH 0091 (National Semiconductor, USA). The frequency range was limited by RC cut-offs at about 0·5 and 10^3 Hz, and the output was averaged with a time constant of 5 s.

Drugs. 4-Aminopyridine (4-AP, Sigma) at 0·5–1 mM was added to the basic Ringer solution. For most experiments involving higher concentrations, [NaCl] in the control solution was reduced to 250 mM and 20 mM-*N*-methyl-D-glucamine (NMDG) was added. Addition of 4-AP was then compensated by reduction of [NMDG]. Tetraethylammonium (TEA) was injected by passing current through a microelectrode containing 1 M-TEACl (Sigma). Many electrodes, especially those through which current was to be passed, were bevelled on a miniature grindstone (De Marco, Switzerland) to a resistance of 30–40 MΩ.

Mathematical modelling. Modified versions of the equations of Hogkin & Huxley (1952) for the space-clamped squid axon membrane were solved on a Sun Sparc computer using Adams' variable step size predictor–corrector algorithm (e.g. Burden & Faires, 1989).

RESULTS

K^+ conductance: effects of 4-AP in darkness and during steady illumination

In the cell of Fig. 2*A*, switching the superfusate to one containing 10 mM-4-AP caused the membrane to depolarize and produce action potentials. After return to normal superfusate, the action potentials ceased and the membrane repolarized again over several minutes. In fifty of sixty-three cells repetitive action potentials were induced by 4-AP at concentrations from 0·5 to 10 mM with the cell in darkness or under steady illumination. Under steady illumination, in the cases where action potentials were not induced, membrane noise increased. These effects were reversible and repeatable. In agreement with other authors we very rarely observed repetitive firing when the cell was depolarized by light in the absence of 4-AP (Baumann, 1968; Coles & Schneider-Picard, 1989*a*). Depolarization of the cell by raising extracellular [K^+] does not induce repetitive firing (Coles & Orkand, 1983; J. A. Coles, unpublished results). 4-AP is a weak base; in its neutral form it readily enters cells and increases intracellular pH (Szatkowski, 1989; Howe & Ritchie, 1991). However, application of 10 mM-NH_4Cl, which is also known to increase intracellular pH, did not induce action potentials (not shown). These observations, and others to be described, are in agreement with the hypothesis that the effects of 4-AP on drone photoreceptors that we observe are due to its usual property of blocking of K^+ channels (Pelhate & Pichon, 1974).

Figure 2*B* shows repetitive action potentials induced by 4-AP when the membrane potential, V_m, was brought to three different values by changing the light intensity. Frequency increased as the membrane was depolarized, and the amplitudes decreased.

Depolarization as a function of membrane potential

To study the depolarization induced by 4-AP without the complication of action potentials, we superfused retinas for two or more minutes with Ringer solution containing 0·5, 1 or 2 μM-TTX and included TTX in the 4-AP solution. As shown

previously, the TTX abolished action potentials (Baumann, 1968; Coles & Schneider-Picard, 1989*a*). The superfusate was then switched to one containing 4-AP either in the dark or when the cell was depolarized by illumination (Fig. 3*A*). The 4-AP induced a depolarization which was maximal after 50–100 s. In the continued

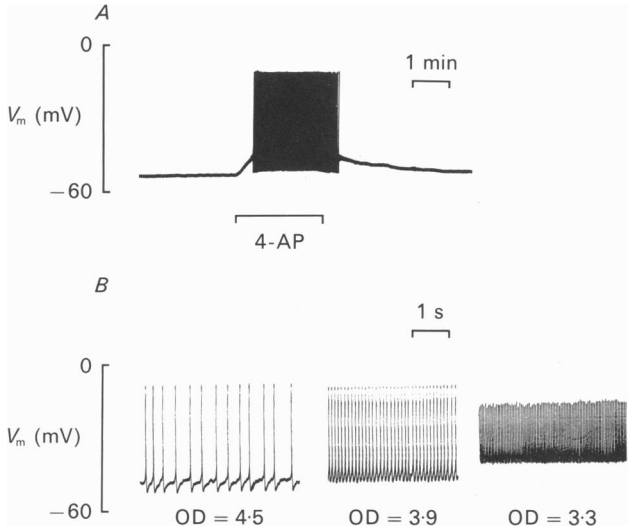

Fig. 2. Typical responses to 4-AP when voltage-dependent Na^+ channels were functional. *A*, in the dark, 4-AP (10 mM) depolarized the membrane and induced repetitive action potentials. *B*, effect of different light intensities on V_m and action potential frequency. The records are from a continuous recording. OD, optical density of attenuating filters.

presence of 4-AP V_m recovered slightly, normally by about 0·5 mV. After removal of the 4-AP V_m returned to within 1–2 mV of its initial value in 5–10 min.

The depolarization induced by 3 mM-4-AP in twenty-four cells is plotted in Fig. 3*B* as a function of the initial V_m set by the intensity of the illumination. The depolarizations produced by 4-AP varied greatly from one cell to another, and in the few cases where more than one application was made at different starting potentials in the same cell no systematic trend was observed. The depolarizations were usually several times greater than those produced by the same concentrations of K^+ (Coles & Orkand, 1983).

We interpret these results to mean that K^+ channels sensitive to 4-AP were open in the steady state over the whole range of V_m that we studied. Little systematic dependence on V_m is apparent among the scatter in Fig. 3*B*. The dashed line shows the depolarization calculated for the circuit shown in Fig. 3*C*. This circuit has a voltage-independent conductance, g_S, with a reversal potential of zero (Baumann, 1974, and below) that represents both the light-activated conductance and a leak conductance, and a voltage-independent K^+ conductance, g_l, that can be partially blocked by 4-AP. E_K, the reversal potential for g_l, is taken as −66 mV, the value that accounts best for the results of Fig. 5*E* (see below). The potential, V, across the circuit is:

$$V = E_K g_l/(g_l+g_S). \quad (1)$$

If 4-AP reduces g_l by a fraction γ, so that its new value is $g_l(1-\gamma)$, then V will depolarize by:

$$\Delta V_\gamma = \gamma\,(V-E_K)/(E_K/V-\gamma). \quad (2)$$

The dashed curve shown in Fig. 3*B* is for $\gamma = 0{\cdot}3$. The dependence of γ on [4-AP] varied from cell to another; the effect had not saturated at 20 mM (11 cells) and the maximum value of γ was 0·8.

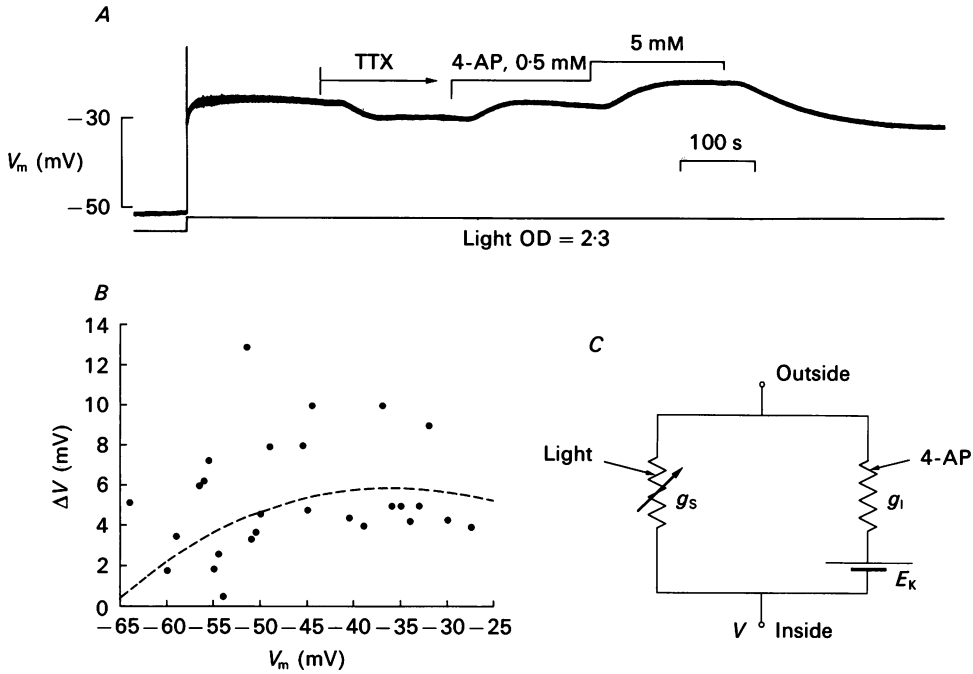

Fig. 3. Effect of 4-AP on V_m in the presence of TTX. *A*, the onset of illumination evoked an action potential (vertical line truncated by the chart recorder). Passage of TTX (1 μM) hyperpolarized the membrane by 3 mV and decreased the noise. 4-AP depolarized the membrane as a function of concentration. *B*, for twenty-four cells, the membrane potential was adjusted with attenuating filters and the depolarization induced by 3 mM-4-AP was measured in the presence of TTX. The dashed curve is calculated from the model circuit of *C* with the assumption that 4-AP reduced the K^+ conductance by 0·3 of its initial value.

Effect of 4-AP on the action potential and the receptor potential

In many types of cell that produce action potentials a delayed rectifier K^+ conductance speeds up the recovery phase, and blocking the delayed rectifier can result in a dramatic lengthening of the action potential (e.g. Armstrong, 1966). The fact that 4-AP induces action potentials at high frequencies (Fig. 2) shows that any possible lengthening of action potentials in drone photoreceptors can only be slight. In Fig. 4*A* the control response is the depolarizing receptor potential evoked by a pulse of light; it has an action potential on the rising phase. When the superfusate was switched to one containing 10 mM-4-AP, V_m depolarized by 6 mV and small action potentials appeared, as described above, but the cell still responded to a pulse of light. In the presence of 4-AP, the receptor potential reached a value more positive

than in the control, and its amplitude measured from the new dark level increased slightly. An increase in membrane resistance due to a reduction in K^+ conductance would have this qualitative effect.

The action potential is shown more clearly in Fig. 4*B*, where the light flash was less intense and the time scale expanded. In drone photoreceptors the steepest part of the

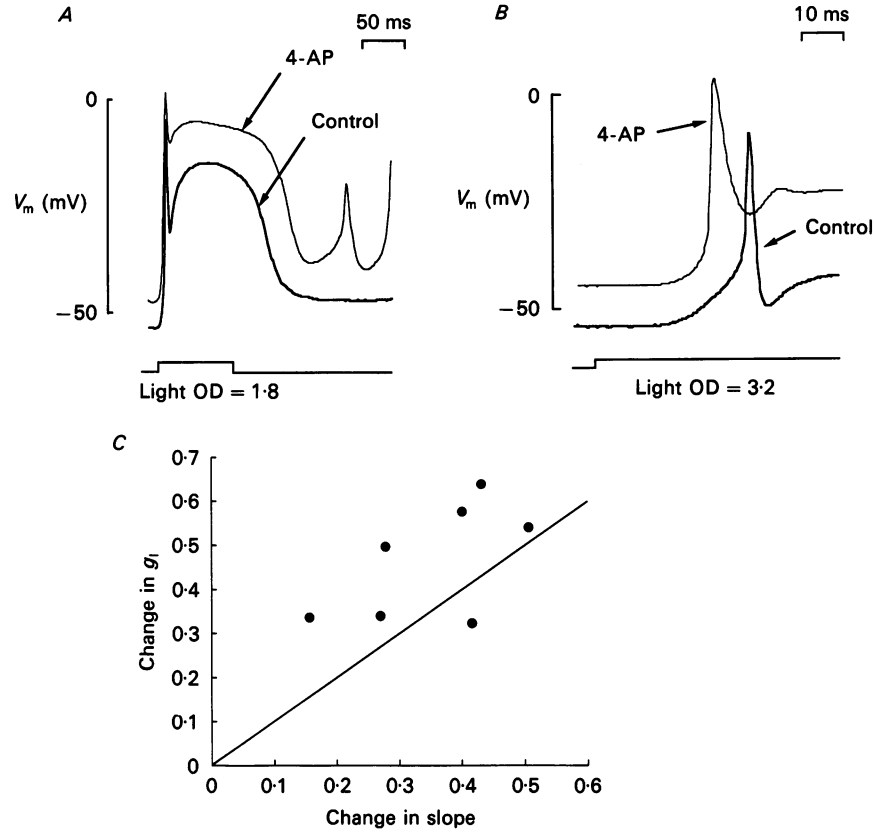

Fig. 4. *A*, effect of 4-AP on the response to a light pulse on a background of darkness. *B*, the action potential at the onset of light. The light stimulus was weaker than in *A*, and the time scale is expanded. *C*, the maximum rate of change of potential on the recovery phase of the action potential was measured before and after application of 4-AP in seven cells. The abscissa is the ratio (slope with 4-AP)/(slope before 4-AP). The ordinate is the fractional decrease in K^+ conductance calculated from the circuit of Fig. 4.

recovery phase of the action potential normally has a slope of about 20 V s^{-1}, which is small compared to the more than 200 V s^{-1} found in the squid giant axon. It is seen in Fig. 5*B* that the recovery was slowed further by 4-AP. The steepest part of the recovery phase was measured before and after application of 4-AP in seven retinas. The ratio (after/before) ranged from 0·16 to 0·51. The steepness of the recovery phase of an action potential is dominated by the rate at which K^+ current recharges the membrane capacitance: in the absence of other conductances the rate of change of voltage would be proportional to the K^+ conductance.

We used the model circuit of Fig. 3*C* to see if the reduction in steady-state K^+ conductance caused by the 4-AP could account for the slowing of the recovery phase, or whether a voltage-dependent conductance was required. For each of the seven retinas, the observed 4-AP-induced depolarization was used to estimate the change in the supposedly voltage-independent K^+ conductance, g_l. In Fig. 4*C*, the calculated change in g_l is plotted against the observed slowing of the recovery phase. For six of the seven cells, the change in g_l was greater than the slowing of the recovery phase; the mean ratio was 1·42 (S.E.M. = 0·17). A ratio > 1 is to be expected since other conductances are involved including a residual voltage-dependent Na^+ conductance, not totally inactivated after the spike, the light-activated conductance, g_S, and the Cl^- conductance (Coles, Orkand & Yamate, 1989). We have not attempted to estimate the effects of these other conductances, but we conclude that if a delayed outward rectifier is present it is either unaffected by 4-AP, or its conductance is small compared to the other membrane conductances.

Responses to small decrements of light intensity

In cells in which 4-AP induced repetitive action potentials we were unable to observe the small responses to small decremental stimuli. When 4-AP did not induce repetitive action potentials (or after they had ceased during wash-out) it increased the amplitudes of the overshoots at the beginning and end of the response (Fig. 5*B*) and in some cases the overshoot at 'off' became a regenerative action potential (Fig. 5*A*). To avoid the complication of these overshoots, TTX was applied and also included in the solution that contained 4-AP. The TTX-resistant response to dimming is a hyperpolarization without overshoots (Coles & Schneider-Picard, 1989*a*; 'TTX' in Fig. 8*A*). As described above (Fig. 3) 4-AP depolarized the membrane, by 3–12 mV in these experiments. Its effect on the response to dimming depended on V_m. When V_m, after application of TTX, but before application of 4-AP, was more negative than about -40 mV, superfusion with 4-AP increased the amplitude of the response to dimming ('4-AP' in Fig. 5*C*). When V_m was more positive than -40 mV, 4-AP decreased the response (Fig. 5*D*). Results from eighteen cells are shown by the filled circles in Fig. 5*E*.

We were concerned that the decrease in the response when V_m was more positive than -40 mV might have been caused, in part, by an effect of 4-AP on the phototransduction. This seems unlikely. In some of the experiments two series of stimuli were given before application of 4-AP and two afterwards and, in most cases, the reduction in response amplitude was complete and stable when V_m reached its new value. That is, the effect of 4-AP on the response to dimming appeared to have about the same time course as its effect on V_m. Prolonged exposure to 4-AP did not reduce the amplitude of receptor potentials produced by light flashes; with $V_m < -40$ mV, 4-AP increased the response. During wash-out of the 4-AP, the response amplitude recovered most of its original amplitude with about the same time course as the recovery of V_m. We did not observe large or consistent effects of 4-AP on the time course of the response with V_m near -38 mV.

We have shown that 4-AP appears to block a non-inactivating K^+ conductance (Fig. 3) and that there is no sign of strong delayed rectification (Fig. 4). Qualitatively, the circuit of Fig. 3*C* can be seen to account for the observed effect of 4-AP on the

responses to the dimming stimuli in the presence of TTX. At negative potentials the main effect of blocking g_l is to increase membrane resistance, and hence increase the effect of a given change in g_S. At more positive potentials the main effect is to bring V_m closer to the reversal potential for the light-activated current, and hence reduce

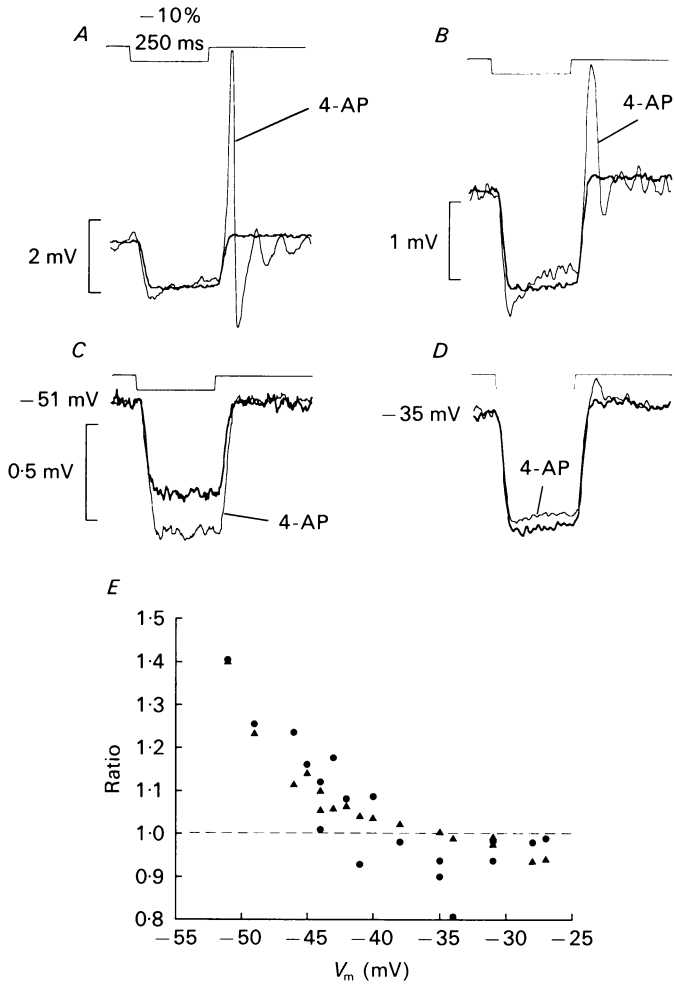

Fig. 5. Effects of 4-AP on responses to a 10% decremental stimulus. *A* and *B*, responses in the absence of TTX. Initial $V_m = -41$ mV, control response shown by the thicker traces. The superfusate was switched to one containing 0·5 mM-4-AP and 130 s later fifteen decremental stimuli were presented; the averaged response (*A*, '4-AP'), understates the amplitude, about 35 mV, of the action potential at the end of each stimulus. Trace shifted downwards by 4 mV to coincide with the control. *B*, response during wash-out of 4-AP when there was no longer a full action potential after the stimulus. *C* and *D*, from two different cells, show responses in the presence of TTX. In *C*, V_m was initially −51 mV and in *D*, −35 mV; the baselines during application of 4-AP have been shifted downwards to coincide with the initial baselines. *E*, a graph summarizing the results. The abscissa is V_m in TTX before addition of 4-AP. The ordinate is the change in peak response amplitude expressed as: (amplitude in the presence of 4-AP)/(amplitude before 4-AP). ●, experimental results; ▲, theoretical values calculated from eqn (5).

the response. To examine the relation quantitatively, we consider the dimming stimulus to reduce g_S by a fraction η to $g_S(1-\eta)$. Then, from eqn (1),

$$\eta = S_1 E_K (E_K V - V^2 + S_1 E_K - S_1 V)^{-1}, \quad (3)$$

where S_1 is the amplitude of the response to dimming.

As for Figs 3*B* and 4*C*, let 4-AP reduce g_l by a fraction γ to $g_l(1-\gamma)$ to depolarize the membrane by ΔV_γ. Then, from eqn (2),

$$\gamma = \Delta V_\gamma \{V(V/E_K + \Delta V_\gamma/E_K - 1)\}^{-1}. \quad (4)$$

The new response to the dimming stimulus, in the presence of 4-AP, is:

$$S_2 = E_K(1-\gamma)\{(E_K/V - \gamma - \eta(E_K/V-1))^{-1} - (E_K/V-\gamma)^{-1}\}. \quad (5)$$

Ratios S_2/S_1 were calculated using the values of S_2 predicted from the observed values of V, S_1 and ΔV_γ and various estimates of E_K. The ratios predicted for $E_K = -66$ mV are shown as filled triangles in Fig. 5*E* and agree well with the observed ratios.

We conclude that in the presence of TTX, the effect of 4-AP on the response to a dimming stimulus can be explained if 4-AP blocks part of a voltage-independent K^+ conductance.

Effects of other K^+ channel blockers

Tetraethylammonium (TEA), which blocks many types of K^+ channels (see Rudy, 1988; Castle, Haylett & Jenkinson, 1989), had no observable effects on drone photoreceptors when included in the superfusate at up to 10 mM. But it had effects that we could not distinguish from those of 4-AP when injected intracellularly by ionophoresis. Superfusion with Ringer solution containing 5 mM-Cs^+ produced depolarizations of 0–3 mV (mean 1·5 mV, $n = 14$, not shown) that reversed on wash-out. At 50 mM Cs^+ produced a depolarization of 3·8 mV ($n = 2$), but this may have been partly due to equimolar replacement of Na^+. The venom of *Bitis gabonica* (Latoxan, France) has been reported to include a component that blocks an inwardly rectifying K^+ conductance in mammalian cardiac muscle (Busso, Camino, Cedrini & Lovisolo, 1988). Its effect on drone photoreceptors was similar to that of 5 mM-Cs^+: at 102 mg l^{-1} it produced reversible depolarizations of 0–4 mV (mean 1·9 mV, $n = 14$). In three tests, switching from 102 to 510 mg l^{-1} caused a further depolarization of 0–2 mV. *Bitis* venom, like 4-AP, increased the overshoots on responses to 10% dimming.

No effects were observed with any of the following agents. Dendrotoxin (Sigma), which blocks certain delayed outward rectifier currents and A-currents, was used at 1 μM. Venom of *Dendroaspis polylepis*, which contains several peptides that block K^+ channels (Harvey & Anderson, 1985) was used at 0·2 mg l^{-1}. Bee venom, which includes apamin, a blocker of Ca^{2+}-activated K^+ channels, was used at 25 mg l^{-1}. R56865, which blocks Na^+-activated K^+ channels in mammalian heart muscle (Luk & Carmeliet, 1990) was used at 1·2–12 μM.

Voltage-dependent Na^+ conductance

Effects of TTX on responses to small changes in light intensity

Figure 6 illustrates the amplification of small signals. A photoreceptor with a membrane potential, V_m, in the dark of −55 mV was depolarized by steady illumination to −38 mV. A series of two decremental and two incremental stimuli (top trace), was presented 100 times and the responses to the series averaged. Under control conditions (trace 1) the responses showed overshoots followed by damped oscillations at 'on' and 'off'. The superfusate was switched to one containing 1 μM-TTX, which caused the membrane to hyperpolarize to reach a steady value of

−40 mV. The stimulus now gave responses that were smaller, slower and without overshoots (trace 2). The effect was not simply due to the hyperpolarization because when V_m was restored to −38 mV by increasing the light intensity the responses remained small and slow (trace 3). Part *B* of Fig. 6 shows single sweeps from the same

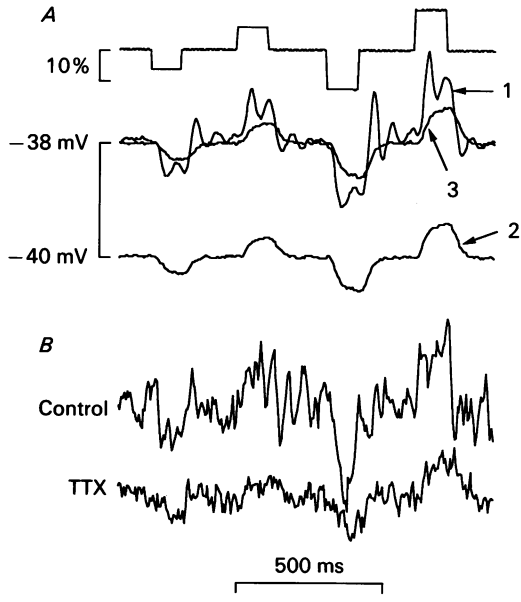

Fig. 6. Intracellular voltage responses to dimming and to brightening. *A*, the top trace shows response of the photodiode to the stimulus. The intensity changes were −6·3, +7·5, −12·6 and +13·0%. Trace 1, the voltage response before application of TTX. Trace 2, after application of TTX. Trace 3, with TTX, light intensity increased by 0·21 log units. In each case 100 responses were averaged. *B*, responses to a single presentation of the intensity changes shown in the top trace of *A*, without averaging, before ('control') and after ('TTX') application of TTX. Voltage scale as in *A* but with arbitrary baselines.

experiment. The noise, whose origin was mainly the fluctuating rate of absorption of photons, was decreased by TTX, as reported by Ferraro, Levi, Lovisolo & Vadacchino (1983). The effect of the TTX took tens of minutes to reverse, and we did not usually obtain stable responses to light modulation over such times, so, as in Coles & Schneider-Picard, 1989*a*, we used a concentration of TTX high enough to have its maximum effect in a short time, less than 2 min, and did not attempt to observe reversal. Doubling or halving [TTX] made no apparent difference.

The time courses of the responses to small decremental or incremental stimuli were variable from one cell to another in that overshoots at 'on' and 'off' could be more or less marked (Figs 6*A* and 8*A*), or completely absent. Responses to dimming stimuli were measured for twenty-five cells with V_m brought to between −38 and −41 mV by the background light. The mean peak-to-peak response, normalized to the amplitude of the dimming stimulus, was 0·90 mV for a 10% dimming (S.D. = 0·44 mV); the greatest response was 2·36 mV. The mean amplitude of the response at 100 ms was 0·67 mV, S.D. = 0·21 mV. This amplitude at 100 ms did not correlate

significantly with the presence of overshoots: a degree of oscillation was expressed by (peak-to-peak response)/(response at 100 ms). The correlation coefficient, R, of amplitude at 100 ms against degree of oscillation was only 0·29. This shows that the overshoots are not an essential part of the amplification.

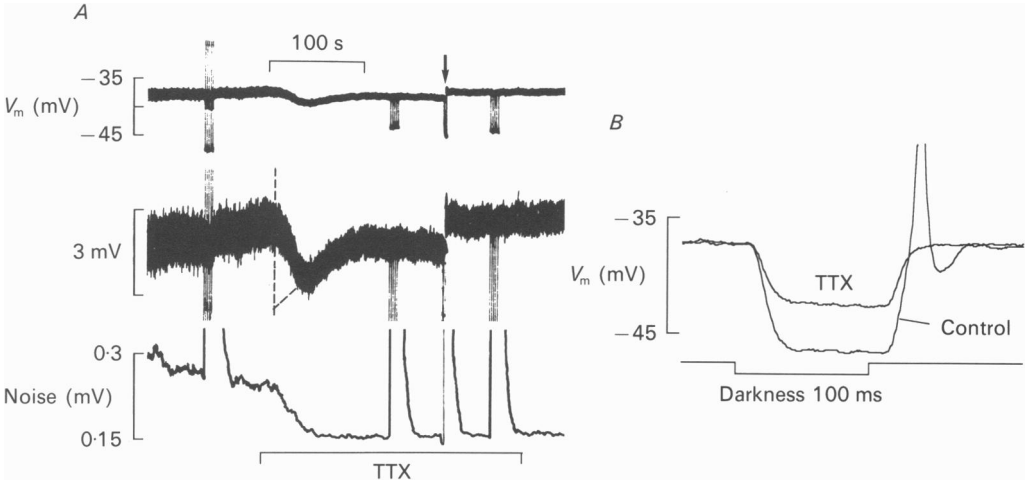

Fig. 7. The effect of TTX on membrane potential and on the response to a pulse of darkness. *A*, chart recording. At three times, five 100 ms pulses of darkness were presented at 2 s intervals; the responses appear as vertical lines in the voltage traces, the positive overshoots being truncated. At the arrow the light intensity was increased. The dashed lines show how the partial recovery from the hyperpolarization was extrapolated back to the time when TTX had its first effect. The bottom trace shows root mean square noise. *B* shows the averaged responses to the first and second series of dark pulses on an expanded scale.

The hyperpolarization caused by TTX and the maximum contribution of the amplification

If the TTX-sensitive component of the negative-going response to dimming is due to the closing, by voltage, of voltage-gated Na^+ channels it follows that the amplitude of this component, at least in the steady state, should not be greater than when all the voltage-gated Na^+ channels closed. To see if this requirement was satisfied, the hyperpolarization in the steady state caused by blocking channels with TTX was compared with the amplitude of the TTX-sensitive component of the response to the greatest possible dimming, i.e. darkness (Fig. 7*A*). The average reduction in amplitude of responses to dark pulses after application of TTX was 2·94 mV, S.D. = 1·30 mV, $n = 8$, which was not significantly different from the mean peak hyperpolarization induced by TTX (2·63 mV, S.D. = 1·47 mV). However, when the TTX-induced hyperpolarization was extrapolated back to the time of the beginning of the deflection (dashed lines in Fig. 7*A*) the extrapolated value (mean 3·8 mV) was bigger than the response reduction by 0·86 mV, S.E.M. = 0·71 mV, $n = 8$. This result is consistent with the interpretation that a sufficient fraction of Na^+ channels are open to support the observed amplification.

A model of the amplification

If the cell membrane is represented by the circuit of Fig. 8*C*, information is available about all the elements except the voltage and time dependence of the Na^+ conductance, g_{Na}.

Total membrane conductance

Carreras (1978) measured a mean time constant of 4·45 ms for the voltage change produced by injection of hyperpolarizing current into a drone photoreceptor in the dark. Because of the electrical coupling between cells (Shaw, 1969) this is probably an overestimate of the membrane time constant. If the specific membrane capacitance, C_M, has its usual value of 1 μF cm^{-2} a lower limit of 0·225 mS cm^{-2} is obtained for the specific membrane conductance. In the version of the model used for Fig. 8*D*, membrane conductance during continuous illumination was 0·320 mS cm^{-2} before application of TTX, and 0·273 mS cm^{-2} in the presence of TTX.

Light-activated conductance, g_S

Whole-cell recordings in *Drosophila* photoreceptors show only weak voltage dependence of the light-activated conductance in the range of interest (-38 mV $\pm$ < 2 mV; Hardie, 1991), and in drone the assumption of weak voltage dependence satisfactorily accounted for the results of Figs 3 and 5. The reversal potential is taken to be zero (Fig. 4.6 in Baumann, 1974; Fig. 2*d* in Hardie, 1991).

Light-induced changes in g_S

The top trace in Fig. 8*A* is the response to a 16·1 % dimming stimulus recorded by an electrode in extracellular space in the presence of TTX. Its time course is an indication of the time course of the stimulus-induced change in the light-induced conductance. This time course is little affected by changes in background light intensity over the range used (Fig. 8*B*). In the model, we mimicked the turn-on and turn-off of the change in g_S in the cell by multiplying g_S by a factor s incorporating a cubic spline:

$$s(t) = 1+\zeta\left\{\frac{3(t-t_0)^2}{(t_1-t_0)^2}-\frac{2(t-t_0)^3}{(t_1-t_0)^3}\right\}, \quad t_0 \leqslant t \leqslant t_1 \tag{6}$$

$$s(t) = 1 \quad t \leqslant t_0, t \geqslant t_1,$$

where ζ is the amplitude of the modulation, t_0 is the beginning of the change in g_S, and t_1 is the beginning of the recovery (top trace in Fig. 8*D*). The steps between absorption of photons and change in g_S are not part of the model. For convenience, the leak conductance is included in g_S; as a consequence g_S is finite in the dark.

K^+ conductance, g_l

The predominant K^+ conductance has negligible voltage dependence in the range of interest and an E_K of -66 mV (Fig. 5). Other values, from -55 to -80 mV, were used in some calculations.

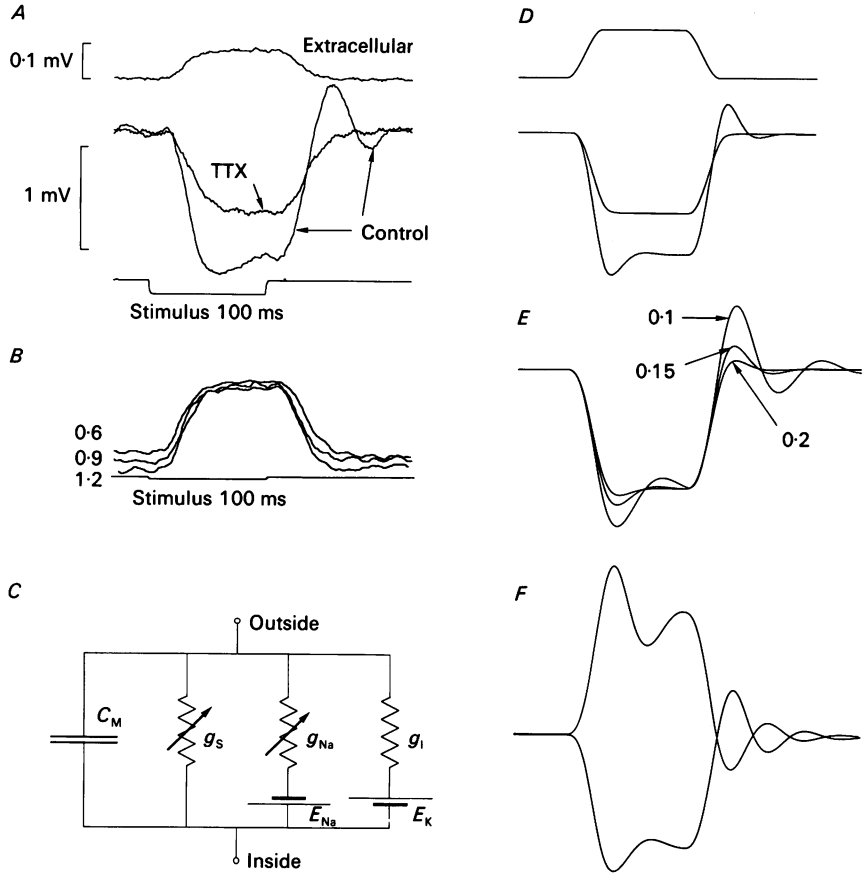

Fig. 8. Responses to a 16·1% dimming stimulus. *A*, the top record ('extracellular') is of extracellular potential in a retinal slice that had been exposed to TTX. The record shown is the average of 200 responses with light attenuation of 0·9 log units. The lower two traces are intracellular responses from a photoreceptor in a different retina. The membrane was depolarized to −38 mV with a background light attenuated by 1·2 log units. A hundred responses to dimming were averaged (Control). TTX was applied and the intensity was increased by 0·3 log units (TTX). *B*, extracellular responses to the dimming stimulus at different background light intensities, after application of TTX. Numbers give light attenuation in log units. The amplitudes have been normalized and the baselines have been shifted so as to partially separate the curves. *C*, the circuit of the model described in the text. Only g_{Na} is voltage dependent. *D*, curves given by the model of *C*. The top trace is the time course, given by eqn (6), of the change in g_S. The parameters were: g_{Na} = 4·0 mS cm^{-2}, g_l = 0·2 mS cm^{-2}, $\zeta = -0{\cdot}045$, k_h = 0·1, $E_K = -66$ mV, $E_{Na} = +57$ mV. V_m was set to −38 mV by adjusting g_S to 0·116 mS cm^{-2}. The smaller response with no overshoots was obtained when g_{Na} was set to zero and g_l was decreased to 0·157 mS cm^{-2} to restore V_m to its original value. *E*, effect of changing the rate of inactivation of g_{Na}. The values of the factor k_h are indicated. Other parameters were: g_{Na} = 3·5 mS cm^{-2}, g_l = 0·17 mS cm^{-2}, $\zeta = 0{\cdot}05$, $E_K = -55$ mV, $E_{Na} = +70$ mV. *F*, comparison of the responses to a decrement ($\zeta = -0{\cdot}05$) and an increment ($\zeta = +0{\cdot}05$) of g_S. The other parameters were the same as those in *D* except that g_{Na} = 4·5 mS cm^{-2}, $E_K = -55$ mV, $E_{Na} = +70$ mV, k_h = 0·15.

Voltage-dependent Na^+ conductance, g_{Na}

During steady illumination at the intensities we used, intracellular sodium activity has been measured to be about 20 mM (see Coles, Orkand, Yamate & Tsacopoulos, 1986), so E_{Na}(light) = +57 mV. In the steady state:

$$V_m = (g_{Na}E_{Na} + g_l E_K)/(g_S + g_{Na} + g_l). \quad (7)$$

When V_m is close to −38 mV, setting $g_{Na} = 0$ should reproduce the 3·8 mV hyperpolarization caused by TTX. $g_{Na}(V_m, t)$ was assumed to obey the equations of Hodgkin & Huxley (1952) for the Na^+ conductance of the squid axon. The best fits obtained with the model were for a resting potential of −55·5 mV, which is a typical resting potential in drone photoreceptors.

The time-dependent membrane potential is given by:

$$0 = C_M \, dV/dt + \bar{g}_{Na} m^3 h(V - V_{Na}) + g_l(V - V_K) + g_S(V - V_S), \quad (8)$$

where g_{Na}, m and h and also the sign convention are defined in Hodgkin & Huxley (1952). In the present paper the figures are labelled with intracellular potential, V_m, referred to the extracellular potential according to the current standard convention.

Responses of the model to small decrements of light intensity

Amplification in the steady state. Satisfactory representation of the responses to small decrements in light intensity was obtained when $\bar{g}_{Na}$ = 4·0 mS cm^{-2} and g_l = 0·2 mS cm^{-2}. To bring V_m to −38 mV, g_S had to be 0·116 mS cm^{-2}; g_{Na} was calculated to be 0·00467 mS cm^{-2} corresponding to 0·00467/4 × 100 = 0·12% of the Na^+ channels being open. The amplitude of the typical observed response of Fig. 8*A* was matched when g_S was decreased by 4·5% to give a steady-state change in V_m of −1·10 mV (larger negative-going response in Fig. 8*D*). To mimic the effect of TTX, g_{Na} was then set to zero. This caused V_m to hyperpolarize by 3·81 mV, in agreement with experiment (Fig. 7*A*). V_m was restored to −38 mV by decreasing g_l to 0·157 mS cm^{-2}. The steady-state response was now −0·725 mV so that the amplification was 1·10/0·725 = 1·52. Not restoring V_m after setting $g_{Na} = 0$, or restoring it by increasing g_S, gave very similar results. We conclude that a Na^+ conductance with a steady-state voltage dependence similar to that of the squid giant axon can amplify small changes in V_m in the steady state.

Overshoots. To produce overshoots like those usually observed at the beginning and end of the responses it was necessary to slow the inactivation of g_{Na} by multiplying the expression for dh/dt by the factor k_h where k_h was in the range 0·1–0·15 (Fig. 8*D*). As shown in Fig. 8*E*, reducing k_h led to bigger overshoots and damped oscillations. The amplitudes of the overshoots varied from cell to cell; in the model, they were reduced when $\bar{g}_{Na}$ and g_l were reduced. The mechanism also amplified small positive changes in V_m (Figs 6 and 8*F*).

The effect of reducing g_l. Application of a low concentration of 4-AP to the cell (Fig. 5*B*) or reduction of g_l in the model by 12·5% both increased the amplitude of the overshoots on the response to dimming. Larger concentrations of 4-AP lead to repetitive firing of action potentials (Fig. 2). Further reduction of g_l in the model also produced repetitive depolarizations although their amplitude was only 9 mV.

Responses to dimming stimuli of greater amplitudes

Figure 9*A* shows responses to dimming of increasing amplitude, before and after application of TTX. Figure 9*C* shows plots of the difference in amplitude of the hyperpolarization before and after application of TTX, as a function of the

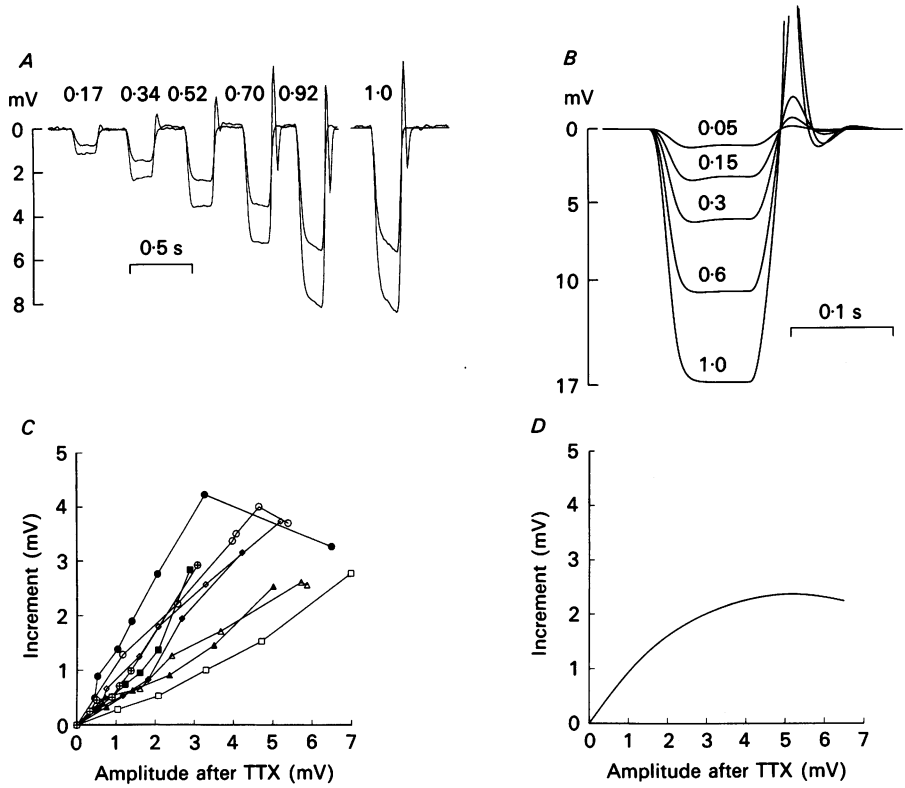

Fig. 9. *A*, a series of dimming stimuli of five amplitudes, increasing from 0·17 to 0·92 of the background intensity (numbers above the record) was presented 50 times and the responses averaged. Dark pulses were then presented (1·0). TTX was applied and the series of stimuli were repeated (responses of smaller amplitude, without overshoots). The large, positive-going overshoots in the control responses were not faithfully recorded. *B*, responses of the model to decrements of g_s by 0·05, 0·15, etc. as marked. The parameters were: $g_{Na} = 3{\cdot}5$ mS cm^{-2}, $g_l = 0{\cdot}17$ mS cm^{-2}, $k_h = -55$ mV, $E_{Na} = +60$ mV. The initial potential was -38 mV. *C*, the TTX-sensitive parts of the hyperpolarizing responses (Increment) in *A* and similar experiments were plotted against the amplitudes of the TTX-resistant responses. Symbols indicate different cells. *D*, a plot, like that of *C*, for the model of *B*.

amplitude after application of TTX. In all of the nine cells, the TTX-sensitive contribution continued to increase approximately linearly for dimming stimuli up to at least 90% (Fig. 9*C*). In the model, as in the real cell, the overshoot on the hyperpolarizing phase disappeared as the amplitude of the dimming increased (Fig. 9*B*). However, in the model the amplitude grew until the hyperpolarizing peak of the

response reached the resting potential (largest response in Fig. 9*B*), whereas in the real cell it did not. A factor contributing to this difference is the depolarizing after-potential observed at termination of illumination (Baumann & Hadjilazaro, 1972; Minke & Tsacopoulos, 1986).

Action potentials. The rapid depolarization at the beginning of the response to a light flash triggers an action potential whose amplitude is typically 55–65 mV. In the model used for Fig. 8*D*, setting g_S so that initially $V_m = -55$ mV and then increasing g_S to 0·25 mS cm^{-2} gave an action potential with an amplitude of 87 mV. The falling phase of the model action potential was slower than that of the real cell; 2.1 Vs^{-1}, compared to about 20 V s^{-1}.

DISCUSSION

Conductances sensitive to 4-AP were present (active) throughout the voltage range -64 to -27 mV (Fig. 3*B*). If these conductances were voltage dependent, the effect of changing the potential was not rapid enough to significantly affect the action potential (Fig. 4*B* and *C*), nor was activation or inactivation apparent in the effect of 4-AP on responses to small changes in light intensity lasting 250 ms in the voltage range -35 to -45 mV (Fig. 5*C* and *D*). On the strength of these observations we developed a simple model to account for the amplitude and time course of the response near -38 mV to a small change in light intensity in which the only voltage-dependent conductance was that for Na^+. With values of g_S, g_l and $\bar{g}_{Na}$ compatible with the other experimental results, the model showed stable amplification of small signals by g_{Na} by factors in the range observed experimentally. With a suitable choice of inactivation time constant for g_{Na} the model would also mimic the overshoots usually observed at 'on' and 'off' of the response to a decremental stimulus (Fig. 8*A* and *D*). Overshoots are not necessary for amplification in the steady state, but they increase the amplification at short times (Figs 5*B* and 6A). Overshoots are increased by application of a low concentration of 4-AP that blocks a small fraction of the K^+ channels, or, in the model, by reduction of g_l; they readily develop into repetitive action potentials (Figs 2 and 5*A*). During continuous illumination in normal Ringer solution repetitive action potentials are occasionally observed, but are thought to be pathological (Baumann, 1968). It appears that drone photoreceptors have evolved to function close to a state of oscillation, so that amplification is close to maximal.

K^+ conductances in drone photoreceptors

Our indirect methods did not demonstrate voltage dependence of the conductance sensitive to 4-AP in the voltage range -64 to -27 mV, but, since nearly all ion channels are voltage sensitive, it would be surprising if the underlying component conductances did not show voltage dependence: this might be outside the voltage range studied, the activation or inactivation might be very slow ($\sim$ 1 s), or there may be several component conductances with different properties. This last idea could explain the finite, but small, effects of Cs^+ and *Bitis* toxin, blockers which are usually specific for inwardly rectifying K^+ conductances (e.g. Mihara, North & Surprenant, 1987; Williams, Colmers & Pan, 1988), and also the failure to fit the dose–response curve for 4-AP with an equation based on a single Michaelis–Menten relation. Certainly, we did not succeed in mimicking certain observed behaviours with the model in which the K^+ conductance was voltage independent. Notably, the action

potential recovered too slowly, and we did not succeed in modelling repetitive firing of full action potentials. Previously described models for repetitive firing appear always to have included a voltage-dependent K^+ conductance, or its phenomenological equivalent (see Jack *et al.* 1975; Chay & Rinzel, 1985). However, even without a voltage-dependent K^+ conductance, the present model gave stable oscillations, although only of moderate amplitude (9 mV) and never of the low frequencies ($\sim$ 1 Hz) that can be observed experimentally (Fig. 2*B*).

The potassium equilibrium potential

Measured values of extracellular $[K^+]$($[K^+]_o$) in superfused slices are close to that of the $[K^+]$ in the superfusate, which is 7·5 mM. During continuous illumination with intensities giving depolarizations to -45 to -30 mV $[K^+]_o$ changes little, but falls rather than rises (Coles & Schneider-Picard, 1989*b*). The best fit of the data in Fig. 5*E* was obtained with $E_K = -66$ mV. From this value of E_K and the value of $[K^+]$ in the superfusate, the free intracellular $[K^+]$, $[K^+]_i$, can be calculated as $[K^+]_i = [K^+]_o \exp(66/25{\cdot}3) = 102$ mM. Direct measurements with intracellular ion-selective microelectrodes gave a value for $[K^+]_i$ of 127 mM in the dark (Coles & Orkand, 1983) with a decrease during continuous illumination with the intensities used of 16 mM (Coles & Schneider-Picard, 1989*b*) so that during illumination $[K^+]_i$ is 111 mM. These measurements were, however, made in retinal slices superfused with Ringer solution containing 210 mM-NaCl, which is less than the 275 mM in the present experiments, and it is possible that $[K^+]_i$ was different. In apparent conflict with the measurements, it is to be expected that $[K^+]_i$ would be lower in the more dilute solution. Fitting the data in Fig. 5*E* with an outward rectifier would have required a more positive value for E_K and increased the discrepancy between this estimate of E_K and that calculated from the concentrations.

Inactivation of the voltage-dependent Na^+ channels

Although steady-state amplification is independent of the kinetics of the Na^+ conductance, to mimic the overshoots usually observed on the small responses we found it necessary to make the inactivation of g_{Na} slower than for the squid giant axon. Such slow inactivation would contribute to the relative slowness of the recovery phase of the action potential observed in drone photoreceptors. Slow inactivation of Na^+ conductances has been found in several different types of nerve and muscle cell (e.g. Connors, Gutnick & Prince, 1982; Muraki, Imaizumi & Watanbe, 1991) and can be produced by activation of protein kinase C (Numann, Catterall & Scheuer, 1991). Since organisms are capable of expressing several different Na^+ channels in different cell types (see e.g. Llinás, 1988), it did not seem useful to compare the supposed Na^+ channel in drone photoreceptors with those from other insect cells.

Comparison of photoreceptors in the drone and the blowfly

Weckström, Hardie & Laughlin (1991) have demonstrated outwardly rectifying K^+ conductances, sensitive to 4-AP, in photoreceptors of another insect, the blowfly, and shown how these conductances could play a major role in light adaptation. The different membrane conductances in photoreceptors of blowfly and drone appear to correlate with the different visual environments in which the two animals function.

The drone spends most of his life inactive in the dark hive where vision would be of little use: in the laboratory, drone photoreceptors are observed to lose, rather than gain, sensitivity during prolonged dark adaptation (Walz, Ziegler, Demmelhuber & Hilbrandt, 1990). The only major activity of the drone is the pursuit of objects resembling queens. This pursuit has been observed to occur against skies whose luminances vary within a range of less than one log unit (A. M. Vallet & J. A. Coles, in preparation). It is therefore reasonable that the drone has a relatively limited capacity for light adaptation and can profit from a voltage-dependent Na^+ conductance that is useful over only a limited range of V_m.

Possible advantages of amplification by a voltage-dependent Na^+ conductance

Although detection of behaviourally important visual stimuli by drones may be limited by the noise inherent in the random fluctuations in photon absorption (Coles & Vallet, 1991) it would be useful to make the signal in the photoreceptors large even if the signal-to-noise ratio were unchanged, because the additional noise introduced in the synapse would then be relatively less. In fact, for brief stimuli, the TTX-sensitive amplification does appear to increase signal-to-noise ratio (Coles & Schneider-Picard, 1989*a*). This may be a consequence of the preferential amplification of a range of frequencies (roughly 15–35 Hz), as apparent in spectral analysis of voltage noise (Ferraro *et al.* 1983) or in the presence of overshoots followed by damped oscillations. If this frequency range were appropriate for the time course of the interesting stimuli, the kinetics of the transduction, and the properties of the synapse to the second-order cells, then the useful signal would be amplified more than the noise.

A large and rapid response to a given change in light intensity might have been achieved in a hypothetical photoreceptor in a different way, by increasing g_l and g_S (to reduce the membrane time constant) and by increasing the change in g_S in response to the stimulus. A disadvantage of this course is that it would require increased membrane currents and therefore increased metabolism. The drone retina has an unusually high oxygen consumption (Tsacopoulos, Poitry & Borsellino, 1981) and is large by insect standards, so the supply of oxygen by diffusion might be a limiting factor. Using g_{Na} to produce transient overshoots would increase the response to a brief change in light intensity with little metabolic cost during maintained illumination.

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