in the patch-pipette solutions, we now show that the synaptic amplification is reduced with light adaptation by the influx of Ca^{2+} through their cGMP-activated channels.

Methods

Whole-cell voltage clamp recordings were obtained from bipolar cells on, or just below, the surface of dark-adapted retinal slices prepared from the retina of the dogfish, *Scyliorhinus canicula*.[5] The slices were continuously superfused with oxygenated Ringer at 16–18 °C, and were viewed under infra-red illumination. The Ringer contained (mM): NaCl 260, KCl 3, $CaCl_2$ 4, $NaHCO_3$ 20, $MgSO_4$ 0.5, urea 350, D-glucose 10, N-2-hydroxyethylpiperazine-N′-2′-ethanesulfonic acid (HEPES) 5 buffered to pH 7.7 when bubbled with 95% O_2 : 5% CO_2. Patch-pipettes were coated with a heated mixture of parafilm, mineral oil and wax to improve gigaseal formation, and when filled had resistances of 2–3 MΩ. K-based patch-pipette solutions contained (mM): KCl 280, $MgSO_4$ 5, HEPES 10, urea 350 buffered to pH 7.3, to which was added 1 mM ATP and 1 mM GTP just before the experiment. Cs-based patch-pipette solution replaced KCl with (mM): CsCl 127, Cs methane-sulphonate 100 and TEA 20. 5 mM Ca^{2+} chelator 1,2-bis(2-aminophenoxy)-ethane-N,N,N′,N′-tetra-acetate (BAPTA) was added to the patch-pipette solutions when required. A computer program (Chelator) was used to calculate the amount of $CaCl_2$ to add to yield a range of free Ca^{2+} concentrations. Following gigaseal formation and subsequent rupture of the membrane patch to establish the whole-cell mode, the dark membrane potential was measured in current clamp. Cells were then voltage-clamped to their dark potentials (which was corrected for the tip potential) and responses to steps of light were obtained as soon as possible before there was any change in the intracellular media, as well as after equilibration. In some experiments retinal slices were stimulated by green test flashes of 20 ms duration from a tungsten lamp positioned below the preparation and this was calibrated by mounting a photodiode in the same position as the preparation. The light absorbed by the rods (rhodopsin molecules bleached per rod (Rh^*)) was estimated from previous ON-bipolar cell measurements in the eyecup,[8] because of self-screening by the rods and the variable thickness of the slice (150–250 μm). The light intensity was adjusted to give half maximal ON-bipolar cell flash responses taken to correspond to one Rh^*, the half-saturation intensity of the voltage response and the b-wave. Much briefer flashes (0.2 or 2 ms) were applied using green L.E.D.'s in intensity-response measurements. For step illumination another green L.E.D. was mounted above the preparation.

Results

Figure 1 illustrates whole-cell voltage clamp recordings obtained from bipolar cells in dark-adapted retinal slices prepared from the virtually all-rod dogfish retina, when no Ca^{2+} chelator was included in the patch-pipette solutions. In the ON-bipolar cell recording (a), application of a light step of about 200 rhodopsins bleached per rod per second ($Rh^* s^{-1}$) induced a large

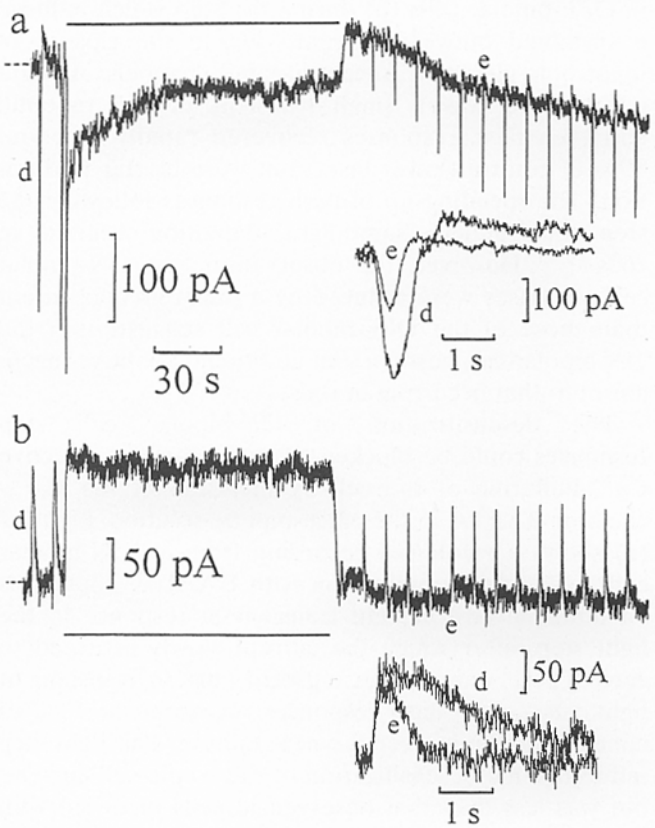

Fig. 1 Desensitization of ON-bipolar cells by steps of light. Whole-cell voltage clamp recordings obtained from bipolar cells in dark-adapted retinal slices, with no Ca^{2+} chelator added to the patch-pipette solution (K-based). Trace (a) is from an ON-bipolar cell voltage-clamped at zero current (dotted lines) to its dark potential (−34 mV) showing inward current responses (downward deflections) to test flashes bleaching 2 rhodopsin molecule per rod (Rh^*). The records begin 30 s after going whole cell. A light step was applied for 2 min, indicated by the horizontal bar above the trace bleaching 200 $Rh^* s^{-1}$. Input conductance measurements were made by applying voltage command pulses at 10 s intervals (not shown) before, during and after the light steps and were 25, 26 and 25 nS, respectively. Similar results were observed in 9 other ON-bipolar cells (n = 10). (b) shows a similar recording from an OFF-bipolar cell, voltage-clamped to its dark potential (−29 mV) obtained 10 min before recording (a) in the same retinal slice. The same light intensities as in (a) were used for test flashes and the light step (horizontal bar below the trace). Input conductances were 13, 12 and 13 nS. (n = 5). Inset records (right) are flash responses shown on expanded timescales before (d) and after (e) the light steps as indicated. (Reproduced with permission from Shiells & Falk, 1999[39])