

transient inward current which was followed by a decline towards zero current during the step. There was negligible change in input conductance between the initial dark levels and during maintained light, suggesting that following the light-induced transient increase in cGMP-activated conductance, the channels rapidly closed again. Following the step exposure, the inward current responses to test flashes were much reduced and had faster timecourses (upper inset) compared with control. No comparable desensitization was observed in OFF-bipolar cells (b) during the step which induced a sustained outward current, due to the closure of ionotropic glutamate receptor-gated channels. At light offset there was a small transient inward rebound and then flash responses recovered rapidly to about 80% of control (lower inset) but were briefer in duration. The speeding up of flash responses following the step would indicate some light adaptation occurring in rods.^{10,11} However, the observation that ON-bipolar cell responses were reduced by a much greater extent than those of the OFF-bipolar cell suggests that the ON-bipolar cell possesses an additional adaptive mechanism to that occurring in rods.

The desensitization of ON-bipolar cell step responses could be blocked by increasing the effective Ca^{2+} -buffering of the cell by inclusion of the Ca^{2+} -chelator BAPTA in the patch-pipette solution. Figure 2 (a) shows a whole-cell recording from an ON-bipolar cell before full equilibration with BAPTA. There was an initial inward current transient in response to the light step, after which the current slowly returned to zero. There was a large outward current transient at light offset. The flash responses recovered to 70% of control amplitude over the next minute. The light step induced some desensitization of the whole-cell current, but was less than that observed in cells recorded with no Ca^{2+} -chelator. Flash responses following the step were also reduced by a smaller extent. On full equilibration with BAPTA (Fig. 2 (b)) there was a small inward shift in the dark current from zero level (dotted lines). The initial rapid transient of the response to the light step was followed by a sustained inward current. Following the light step, flash responses recovered to control amplitude within 30 s, then increased to 125% of control amplitude over the next minute. Outward current rebounds were observed on the flash responses with BAPTA and were more pronounced following the step exposure. The rapid buffering of the Ca^{2+} influx by BAPTA ($\tau = 17 \text{ msec}^{12}$) was effective in blocking desensitization of the sustained component of the step response and adaptation of flash responses. The rapid timecourse of ON-bipolar cell desensitization by Ca^{2+} influx was illustrated by superimposing scaled step responses in the absence and presence of BAPTA (inset, Fig. 2). The rising phase of the responses super-

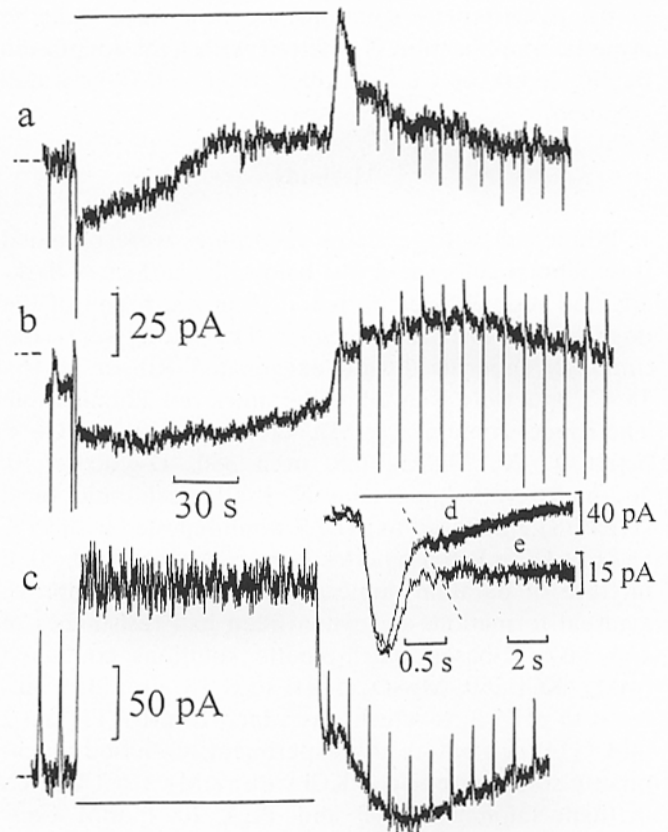


Fig. 2 Desensitization of ON-bipolar cell step responses is blocked by BAPTA. (a) Whole-cell recording from an ON-bipolar cell before full equilibration with 5 mM BAPTA in the patch-pipette solution (K-based). The record begins 3 min after going whole-cell, with the cell voltage-clamped to its dark potential (-28 mV) at zero current (dotted lines). Two control responses to 1 Rh^* flashes were recorded and then a $100 \text{ Rh}^* \text{ s}^{-1}$ step was applied for 2 min, indicated by the horizontal bar above the trace. The input conductance was 22, 26 and 23 nS in darkness, at the beginning and after the light step respectively. (b) Continuation of the same whole-cell recording on equilibration with BAPTA. The record begins 11 min after going whole-cell with the test flash intensity increased to 2 Rh^* and the light step intensity was $200 \text{ Rh}^* \text{ s}^{-1}$. Input conductances were 24, 26 and 22 nS. ($n = 5$). (c) Similar recording from an OFF-bipolar cell with 5 mM BAPTA in the patch pipette solution, voltage-clamped to its dark potential (-52 mV). The test flash intensity was 2 Rh^* and the step bleached $200 \text{ Rh}^* \text{ s}^{-1}$, indicated by the horizontal bar below the trace. Input conductances were 15, 13 and 15 nS. ($n = 5$). The inset shows the initial transient of the response to a step on a rapid timescale. Records (d) without BAPTA and (e) with BAPTA in the patch-pipette solution are scaled to the same peak amplitude and superimposed. They are shown on an expanded timescale initially, and then on a reduced timescale (timescale change indicated by the dotted line). (Reproduced with permission from Shiells & Falk, 1999³⁹).

imposed, whilst the response in the absence of BAPTA (d) decayed earlier and the current desensitized to near zero within 5 s. With BAPTA (e) the sustained inward current showed no desensitization. Only sustained step responses were observed in the OFF-bipolar cell