step offset there was a small outward transient followed by recovery of flash response sensitivity to control. With the ten times brighter background (b), only very small responses could be obtained on increasing the test flash intensities to much higher levels, indicating a longer lasting and more profound desensitization. Similar results were obtained with patch-pipette solutions containing BAPTA nearly-saturated with Ca^{2+} at 50 μM free Ca^{2+}.

When the same experiment was repeated on an ON-bipolar cell equilibrated with BAPTA with no Ca^{2+} in the patch-pipette solution (Fig. 5 (a)), no desensitization was observed. This cell initially showed desensitization to steps just after membrane rupture, and the influx of BAPTA induced an increase in inward current shown as a displacement from zero current level. The dim light step induced a sustained inward current, and the reduction in peak flash response amplitude could be accounted for by superposition of flash responses in the dark onto the background shift in current induced by the step. With the brighter 10 $Rh^* s^{-1}$ background (Fig. 5 (b)) BAPTA blocked the much more profound desensitization observed with the same background (Fig. 4 (b)). The flash responses superimposed on the step showed a marked change in waveform with the appearance of large outward current rebounds, also observed in flash responses following bright steps (Fig. 2 (b)) with BAPTA. OFF-bipolar cells also showed no initial suppression of the test flash response with dim backgrounds (Fig. 5 (c)), and the reduction in peak amplitude could be accounted for simply by superposition onto the shift in current induced by the background illumination. The reduction in their dim light response amplitude was only by about 10%, confirming no significant reduction in rod sensitivity with the 1 $Rh^* s^{-1}$ background.

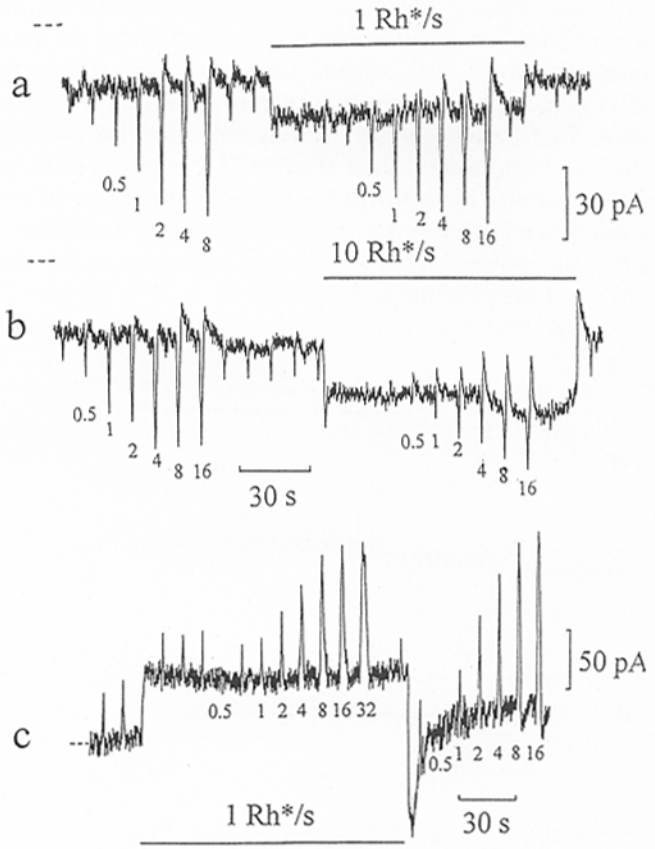

Fig. 5 Absence of desensitization of ON-bipolar cell flash responses against dim backrounds with BAPTA included in the patch-pipette solution. (a) Whole-cell recording from an ON-bipolar cell, voltage clamped to its dark potential (−36 mV), on full equilibration with 5 mM BAPTA in the patch-pipette solution (Cs-based). The record begins 10 min after membrane rupture, and the intensity-response relation was initially determined in the dark by the application of 0.2 ms light flashes of intensities indicated below the inward current responses. A dim light step bleaching 1 $Rh^* s^{-1}$ was then applied for the duration indicated by the horizontal bar. The intensity-response relation was again determined in the presence of the background. (b) This protocol was repeated on the same cell, superimposing test flashes onto a brighter (10 $Rh^* s^{-1}$) background. Large outward current rebounds were observed on the flash responses superimposed on this background. Input conductance increased from 31 nS in the dark to 36 nS with the step in (a) and from 28 nS to 33 nS in (b) respectively. Inward current shifts from zero level (dotted lines at the beginning of the records) were induced by BAPTA. (n = 3). (c) Similar recording from an OFF-bipolar cell voltage clamped to its dark potential (−39 mV), with no Ca^{2+}-chelator included in the patch-pipette solution (K-based). The intensity-response relation was determined against a dim background step (1 $Rh^* s^{-1}$) and then in the dark. (n = 3). This recording was obtained in the same retinal slice as the ON-bipolar cell illustrated in Fig. 4. (Reproduced with permission from Shiells & Falk, 1999[39]).

Discussion

The results show clear dependence of ON-bipolar cell light responses on the degree of intracellular Ca^{2+}-buffering. Desensitization, or adaptation, of light responses was blocked by the inclusion of BAPTA in the patch-pipette solutions. Consistent with these results, isolated ON-bipolar cell current responses did not desensitize to concentration-jumps of glutamate when EGTA was used as the intracellular Ca^{2+} buffer.[3] OFF-bipolar cells gave sustained responses to steps of glutamate and to steps of light which produced profound desensitization of ON-bipolar cells. Consistent with desensitization occurring in their ON-bipolar cell but not OFF-bipolar cell inputs, adaptation with dim light was observed in ON-ganglion cells of frog and cat retina but not in OFF-ganglion cells.[15,16] The results suggest that during light adaptation ON-bipolar cell dendrites receive a Ca^{2+}-load, due to the opening of cGMP-activated channels whose conductance is not reduced by external Ca^{2+}.[17] Relatively high concentrations of Ca^{2+} (20–50 μM) were required to reduce