

Role of Intracellular Calcium and Sodium in Light Adaptation in the Retina of the Honey Bee Drone (*Apis mellifera*, L.)

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ABSTRACT In the honey bee drone, the decrease in sensitivity to light of a retinula cell exposed to background illumination was found to be accurately reflected by the difference in amplitude between the initial transient depolarization and the lower steady depolarization evoked by the background light. It is shown that both the decrease in sensitivity to light and the accompanying drop in potential from the transient to the plateau can be prevented by injecting EGTA intracellularly. A decrease in duration and amplitude of responses to short test flashes such as observed immediately after illumination was found to occur too when Ca or Na, but not K, Li, or Mg, were injected into dark-adapted retinula cells. Injection of EGTA into a retinula cell maintained at a steady state of light adaptation, was found to cause an increase in amplitude and duration of the response to a short test flash, thus reproducing the effects of dark adaptation. It is suggested that, in the retina of the honey bee drone, an increase in intracellular calcium concentration plays a central role in light adaptation and that an increase in intracellular sodium concentration, resulting from the influx of sodium ions during the responses to light, could lead to this increase in intracellular free calcium.

INTRODUCTION

Light adaptation, the decrease in sensitivity of a visual cell exposed to light, has been observed both in vertebrate and in invertebrate eyes. In the vertebrate retina, Dowling and Ripps (1972) found that there are at least two different mechanisms of light adaptation, both of which affect the receptor cell. One of them is related to the bleaching and resynthesis of photopigment, while the other, less well known and sometimes called the receptor process, is independent of changes in photopigment concentration.

Regarding invertebrates, a study of the *Limulus* ventral eye has shown that the photopigment concentration is restored to the dark-adapted value much more rapidly than is the sensitivity to light. It would seem, therefore, that in *Limulus* ventral eye, the receptor process plays an important role in light adaptation (Fein and De Voe, 1973).

It has recently been observed that sodium and calcium ions might be involved in visual adaptation. In *Limulus*, when injected intracellularly, they were found to reduce the responsiveness to light of the ventral photoreceptor (Lisman and Brown, 1972); in the honey bee drone, intracellular injection of sodium was found to cause changes in the shape of the receptor potential similar to those observed immediately after exposure to a background light (Baumann, 1972).

In the present study on the photoreceptor of the honey bee drone, the effects of changes in the intracellular concentration of sodium and calcium and of light adaptation on the shape of the receptor potential were examined. The results indicate that changes in the intracellular concentration of sodium and calcium are involved in and may be the determining mechanism of light adaptation of the retinula cell of the honey bee drone.

METHODS

The retina of the honey bee drone (*Apis mellifera*, L.), prepared as described previously (Baumann, 1968), was placed in a Lucite chamber continuously perfused with oxygenated Tris-Ringer solution of the following composition (mM): Na⁺, 280; K⁺, 3.2; Ca⁺⁺, 1.8; Cl⁻, 287; Tris HCl, 9.0; glucose, 10; pH, 7.3; temperature was maintained at 25°C.

Stimulation

Light emitted by a 150-W Xenon lamp (spindler and Hoyer, Göttingen, Germany) was focused on a diaphragm which could be occluded by an electromechanical shutter. The light beam was then collimated and the image of the diaphragm focused on the retina with a microscope objective. The diameter of the illuminated area was about 800 μm and included the whole length of an ommatidium. The retinal irradiance of the unattenuated beam was 100 $\mu\text{W}/\text{cm}^2$. The light stimulus was monitored with a photocell. Light intensity was controlled by calibrated neutral density filters and was measured on a logarithmic scale, taking as unity the intensity of the unattenuated light. When background light was needed, a second beam from the same source was used. The two beams were reunited by means of mirrors.

Recording

Intracellular potentials were recorded with micropipettes (Corning 7740, Corning Glass Works, Corning, N. Y.), filled either with 3 M KCl or with one of the solutions to be injected. The DC resistance of the pipettes filled with 3 M KCl was between 10 and 40 M Ω . Recordings were made using standard amplification equipment. The positions of the light and of the micropipette were controlled by stereomicroscope.

Injection

Intracellular injections were made through the same micropipette used for recording membrane potential. Injection was performed either by iontophoresis or by using a pressure device described in detail in a previous paper (Bader et al., 1974). For pressure injection the pipette was connected to a metal tube containing alcohol which could be heated by passing a current through a platinum wire. Heating of the alcohol caused the pressure in the tube to rise thus forcing the solution contained in the pipette into the cells. Pressure injection was monitored by applying short current pulses through the intracellular micropipette. The voltage drop across the resistance of the micropipette induced by these pulses was balanced out using a bridge circuit before pressure was applied so that the remaining deflection visible on the oscilloscope reflected only the resistance and the

capacitance of the membrane of the visual cell. The resistance of the pipette is determined partly by the conductivity of the solution it contains and partly by the conductivity of the solution surrounding the tip. The latter solution, if of lower conductivity than the solution to be injected, might dilute the solution in the tip of the pipette. When pressure is applied the undiluted solution is forced into the tip of the pipette and into the cytoplasm. This decreases the resistance of the pipette and upsets the balance of the bridge which does not recover until some time after the heating current has been turned off. Since it takes time for the pressure device to cool, the solution continues to be forced into the cell even when the heating current is discontinued. Consequently, the resistance of the pipette remains lower than it was before the injection and does not recover until the solution in the tip of the pipette reaches its initial conductivity.

The injected solutions were: 3 M KCl; 2 M NaCl; 3 M LiCl; 2 M MgCl₂; 0.1 M CaCl₂ in 3 M KCl; CaEGTA and EGTA. CaEGTA solutions were prepared by combining the following two solutions in different proportions: (solution A) EGTA, 70 mM; CaCl₂, 70 mM; KCl, 300 mM; Tris, 100 mM; pH adjusted to 7.2, using 1 M KOH; (solution B) EGTA, 70 mM; KCl, 300 mM; Tris, 100 mM; pH 7.2. A given calcium concentration can be obtained by varying the amounts of both solutions in the mixture. The free calcium concentration in the solution can be calculated from the equation:

$$[\text{Ca}^{++}] = |\text{CaEGTA}|/|\text{EGTA}| \times 1/K',$$

(K' , the combined apparent association constant of EGTA for calcium, is 1.2×10^7 at pH 7.2 [L. Girardier, personal communication].)

RESULTS

Effect of Background Light on Responses to Superposed Flashes

The visual cells of the honey bee drone, like those of other invertebrates and of vertebrates as well, decrease in sensitivity to light when the retina is exposed to a background illumination. This decrease, as shown by the results of the following experiments, occurs with a certain delay after the beginning of the background illumination, and is probably related to changes in membrane potential which take place during the illumination of the visual cells with the background light.

In the experiment illustrated in Fig. 1, a short test flash was applied either before exposure to background lights of two different intensities, or superposed on them with two different delays. The response to the background light alone was an initial transient depolarization followed by a steady depolarization of lower amplitude. The response to the short test flash was a large transient depolarization when the flash was applied to the dark-adapted preparation (first response in each row) and a depolarization of lesser amplitude when the test flashes were superposed upon the initial transient or the steady-state component of the response to the background light. The level of the membrane potential to which the retinula cell was depolarized at the peak of the response to the late (third) test flash was much lower than that reached at the peak of the response to the early (second) test flash.

The last response in each row was obtained by applying to the dark-adapted preparation a flash whose intensity was equal to the sum of the intensities of the background and of the test flash. This flash was found to depolarize the cell to the same level as did the early test flash but to a higher level than did the late test

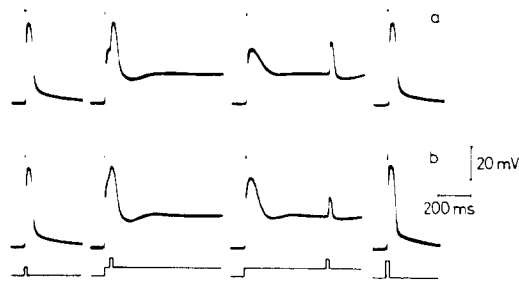


FIGURE 1. Recordings of responses to short test flashes applied before and during a lasting background illumination. In the experiments illustrated in rows *a* and *b*, a test flash of 20-ms duration was applied first before and then superposed upon a background light 35 and 500 ms after the beginning of the background. The background light of 1-s duration was applied at a rate of 1/10 s. The last recording in both rows is the response of the dark-adapted cell to a single flash the intensity of which was equal to the sum of the intensities of the background and the test flash. In row *a* the intensity of the background was -2.8 and that of the test flash -2.2 ; in row *b* these intensities were, respectively, -2.6 and -2.2 . The dot at the beginning of each response is the peak of a spike potential appearing during the rising phase of the receptor potential. The light stimuli are shown in the bottom trace.

flash. It would seem therefore that at the beginning of the background illumination the sensitivity of the cell, expressed as the level of membrane potential to which a cell is depolarized by a given light intensity, was as great as that of the dark-adapted cell and that the decrease in sensitivity due to the background illumination occurred during the interval between the early and the late test flash. That light adaptation takes place during this interval is further indicated by the reduction in the duration of the response to the late test flash. The reduction in amplitude of the response to the test flashes, i.e. depolarization reached at the peak minus depolarization evoked by the background, which occurred as soon as the background light was turned on, is not due to a decrease in sensitivity of the visual cell but is simply the expression of the logarithmic relationship between the amplitude of a visual response and the intensity of the stimulating light in the dark-adapted preparation.

It is important to note in Fig. 1 that the amplitude of the responses to the test flashes superposed on the background light remained relatively constant regardless of the delay. This indicates, as already suggested by Naka and Kishida (1966), that if the potential change evoked by the background had not dropped from the initial transient to the plateau, the peaks of the responses to the test flashes would have reached approximately the same level of depolarization and there would have been no decrease in sensitivity of the retinula cell. The drop in receptor potential from the initial transient to the plateau might, therefore, be a reflection of light adaptation.

The relation between the drop in receptor potential from the initial transient to the plateau and the decrease in sensitivity is illustrated graphically in Fig. 2*a*. In this experiment, flashes of 100-ms duration were applied to the retina at intervals of 30 s, each having an intensity twice that of the previous flash. A series

of these flashes was applied first on the dark-adapted retina and then superposed on backgrounds of increasing intensities. Sufficient time was allowed after each increase of background intensity to permit stabilization of the membrane potential. The total potential change induced by stimulation with a flash or with both a background light and a flash was plotted as a function of the logarithm of the total light intensity of the stimulation.

Except in the case of very weak or very strong flashes, the amplitude of the receptor potential of the dark-adapted preparation increased linearly with the log of the flash intensity. A similar relationship between potential change and light intensity could be observed when flashes were superposed on weak backgrounds, i.e., light depolarized the cell to the same level whether applied in a single flash or subdivided into a lasting background and a short flash. For backgrounds of stronger intensity, however, the total depolarization induced by a given intensity of light was reduced when this intensity was subdivided into a lasting background and a short flash. The amplitude of the response to the test flashes could still be seen to increase linearly, and the slope of the curves relating the amplitude of the visual response to the log of light intensity was the same as that observed in the dark-adapted preparation.

Transient and steady-state potential evoked by the backgrounds applied on the dark-adapted preparation were measured too, and are represented in Fig. 2 *a* by the large open circles. A comparison of the potential difference between the transient and steady depolarization evoked in the dark-adapted preparation by a light stimulus with the number of millivolts by which a stimulus-response curve was shifted downward on the Y axis when background light of the same intensity was used, indicated that the Y axis shift corresponds almost exactly to the potential difference between the transient and plateau of the dark-adapted response. It therefore follows, that if the stimulus-response curves obtained in the presence of a background light were shifted upward by the number of millivolts corresponding to the potential difference between transient and steady state, all the points would coincide with those of the stimulus-response curve established for the dark-adapted preparation. Any loss in sensitivity induced by background lights would thus be cancelled out. This upward shift would also cancel out the shift of the stimulus-response curves along the X axis. The latter is a quantitative measure of the decrease in sensitivity of a visual cell exposed to an adapting light, if the loss in sensitivity is expressed by the amount by which the intensity of the test flash must be increased to produce a response equal in magnitude to the dark-adapted control (Goldsmith, 1963).

This finding lends support to the suggestion that the drop in membrane potential from the transient to the plateau is closely related to the decrease in sensitivity of a visual cell. This is further supported by the fact that no significant loss of sensitivity was observed when the retina was exposed to weak backgrounds (-3.0 and -2.4) which induced a steady-state depolarization only.

EFFECTS OF INTRACELLULAR INJECTION OF EGTA It has been found that in the honey bee drone, the difference in amplitude between the transient and the plateau is considerably reduced when the calcium concentration of the bathing medium is decreased and is more marked when this concentration is increased

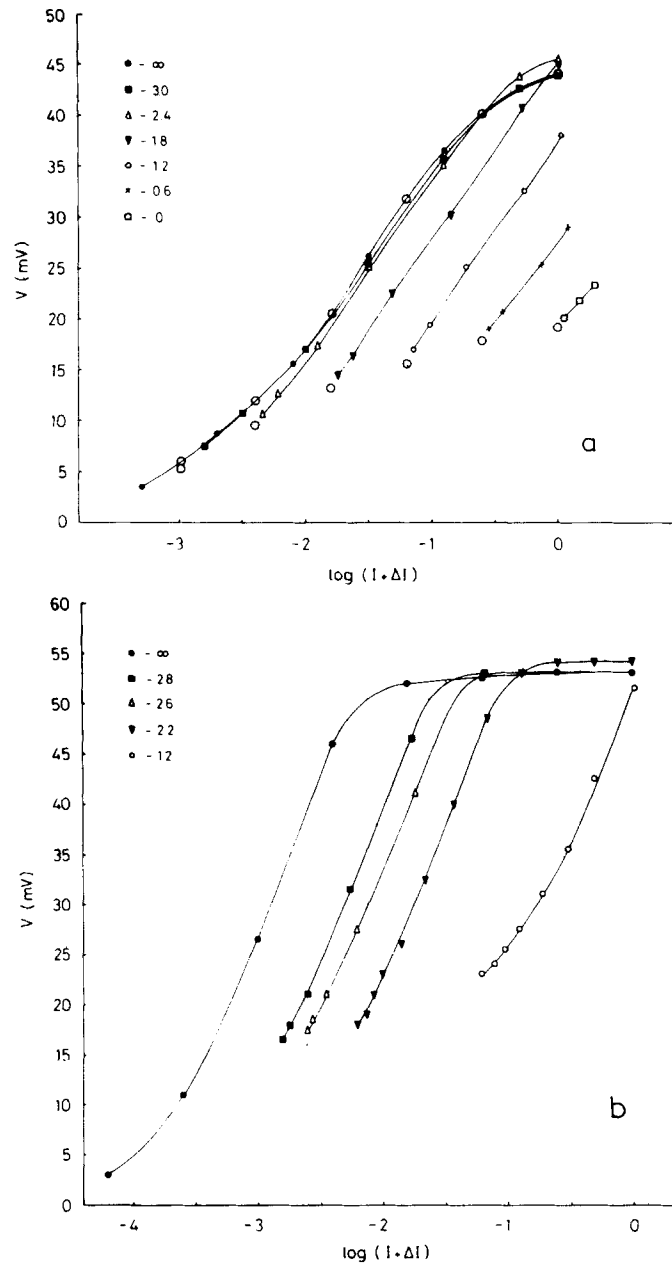


FIGURE 2. Effects of background lights on the stimulus-response curve. After determination of a control curve (closed circles) in which the amplitude V of responses to flashes was plotted versus the \log_{10} of flash intensity ΔI , the flashes were superposed on background lights of increasing intensity each represented by a different symbol. The total potential change V evoked by the flash and background light was plotted versus the \log_{10} of the sum of the two light intensities ($I + \Delta I$). The flashes of 100-ms duration were applied at a rate of 1/30 s. The large open circles in

(Fulpius and Baumann, 1969). Calcium ions may therefore be involved in a sequence of events controlling the drop of the membrane potential from the transient to the plateau and thus be a determining factor in the control of changes in sensitivity occurring in visual cells during background illumination.

In order to test this hypothesis, retinula cells were injected with EGTA in an attempt to reduce the intracellular free calcium concentration and to prevent the changes in this concentration which illumination might induce. In these experiments the retina was stimulated regularly with long-lasting flashes. After a control response was recorded (Fig. 3 *a*), EGTA was injected by pressure into the impaled cell. This caused a slight increase in the amplitude of the transient and completely prevented the drop in membrane potential to a lower plateau (Fig. 3 *b*). The duration of the response to light was considerably prolonged; instead of dropping abruptly at the end of the flash, the membrane potential slowly returned to the dark potential and very often presented a hump similar to that observed in the absence of EGTA in retinula cells illuminated with very strong and long-lasting light stimuli (Baumann and Hadjilazaro, 1972). Responses to light recovered completely approximately 2 min after a single injection of EGTA (Fig. 3 *c*).

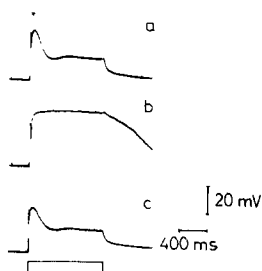


FIGURE 3

FIGURE 3. Effects of intracellular injection of EGTA on the receptor potential evoked by a long-lasting flash. The preparation was stimulated with a flash of constant intensity at a rate of one flash per 15 s. EGTA was injected by pressure (heating current 2.4 A; duration 3 s). *a* is the response recorded before the injection of EGTA; *b* and *c* are the responses recorded 30 and 135 s, respectively, after the end of the heating current.

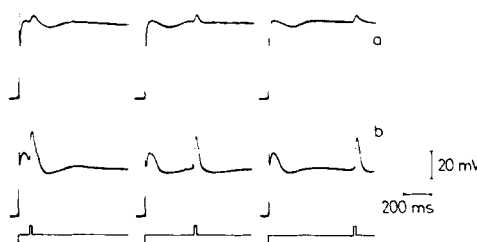


FIGURE 4

FIGURE 4. Recordings of responses to short test flashes applied during a lasting background illumination in a cell injected with EGTA. The cell was impaled with a pipette which leaked EGTA. A 20-ms test flash was applied to the cell 80, 360, and 620 ms after the beginning of a background light of 1-s duration. The intensity of both the background and of the test flash were -1.2 . In *a*, the background light was applied at a rate of one per 30 s and in *b* at a rate of one per 5 s.

a represent, for all background intensities, the potential measured at the peak of the transient and during the plateau of the response to the background. A 6-V tungsten filament served as the light source in *a* and a 150-W Xenon arc which permitted one to apply stimuli sufficiently strong to saturate the receptor potential was used in *b*. The curves in *b* were obtained from the same experiment as that illustrated in Fig. 1.

In some cells, an EGTA concentration which prevented the drop in membrane potential from the transient to the plateau could be maintained for a long time. This was possible because some of the pipettes leaked EGTA spontaneously. In eight cells test flashes were superposed on the background light with different delays. All of them depolarized the cell to the same potential level (Fig. 4 *a*). Often, however, the duration of the response to the second and third test flashes was slightly shorter than that of the response to the early test flash. This could be explained by an intracellular EGTA concentration too low, and a buffering capacity insufficient, to prevent a slight increase in intracellular calcium concentration during the background illumination.

Neutralization of light adaptation by EGTA was reversible. When the frequency of stimulation with the background light was raised (Figs. 4 *b* and 5 *c*) or the intensity of the background increased (Fig. 5 *b*), the response to the background was once again a transient depolarization followed by a plateau of lesser amplitude. The maximum level of depolarization of the response to the late test flash was lower than that of the response to the early one, indicating that the reduction in sensitivity due to the presence of the background light was no longer prevented. When the cells were once again stimulated with a lower intensity or at a lower frequency, light adaptation was again neutralized. It is seen in Fig. 5 *c* that the response to the late test flash reached a maximum level of depolarization lower than that of the response to the early one; its amplitude, however, was much greater. This can be explained by the fact that the amplitude of the receptor potential has a tendency to become saturated when a retinula cell is exposed to high intensity stimuli (Fig. 2 *b*). Thus, since the transient of the response to a high intensity background would be in the saturation region, the amplitude of the response to a superposed flash would have to be low. After the membrane potential has dropped from the transient to the plateau, however,

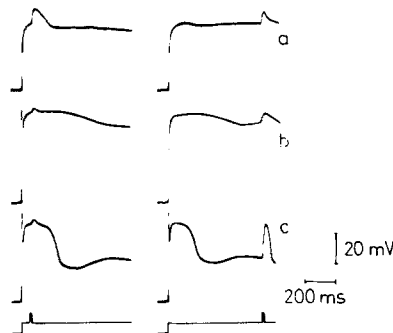


FIGURE 5. Effects of a change in intensity and frequency of a background illumination on responses to light in a cell injected with EGTA. The cell was impaled with a pipette that leaked EGTA. A 10-ms test flash was applied 50 and 620 ms after the beginning of the background light. In *a* and *b* the background light was applied at a rate of one per 30 s and in *c* at a rate of one per 10 s. In *a*, the intensities of both the background and of the test flash were -2.4 ; and *b* and *c*, they were -1.5 for the background and -1.2 for the flash.

and is no longer in the saturation region, the range within which the response to a test flash superposed on the background can be expressed is extended. The small amplitude of the responses to late test flashes in cells containing EGTA and stimulated at low frequencies (Figs. 4 *a* and 5 *b*) would therefore be due to the fact that the membrane potential remained in the saturation region for the duration of the background light.

Aftereffects of Background Illumination

It has been shown previously (Baumann, 1968) that not only do changes in the amplitude and the duration of responses to flashes occur when flashes are superposed on a background light but that they also subsist for some time after the background light is extinguished. Changes in duration of the receptor potential after exposure to a background or adapting light are more marked and last longer than changes in amplitude, and therefore the aftereffects of background illumination can best be observed in response to brief test flashes recorded with a fast sweep speed. Aftereffects of background illumination on responses to test flashes are illustrated in Fig. 6. In this experiment, the preparation was stimulated regularly with flashes of constant intensity and duration. After recording of a control response (Fig. 6 *a*), the cell was exposed to a weak adapting light and two responses were recorded 10 s (fig. 6 *b*) and 80 s (Fig. 6 *c*), respectively, after the adapting light had been turned off. It is seen that the adapting light reduced the duration of the receptor potential and that the

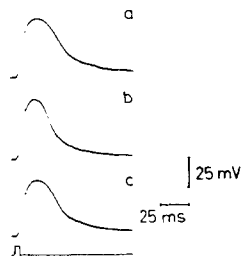


FIGURE 6

FIGURE 6. Aftereffects of illumination. The preparation was stimulated regularly with flashes of 5-ms duration at a rate of one flash per 10 s. *a* is a control response. *b* is the response to a flash recorded 10 s after the end of steady adapting light of 10-s duration whose intensity was the same as that of the flash. *c* was recorded 80 s after the end of the adapting light.

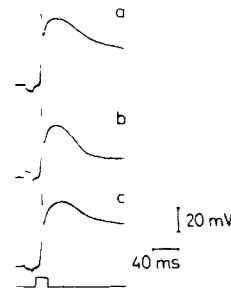


FIGURE 7

FIGURE 7. Effect of intracellular injection of CaEGTA on the response to a short flash. The preparation was stimulated regularly with flashes of 20-ms duration at a rate of one flash per 10 s. A CaEGTA solution with a calculated free Ca concentration of 3.3×10^{-6} mol/liter was injected into the cell by pressure (heating current 2.3 A; duration 10 s). *a* is the response to the flash recorded before injection; *b* and *c*, the responses recorded 30 s and 6 min, respectively, after the end of the heating current. The negative deflection before the onset of the receptor potential is the membrane response to a hyperpolarizing current pulse applied to the micropipette. The bridge was balanced in *a*; in *b* it can be seen that the balance is upset indicating that the solution is being forced into and out of the tip of the pipette.

response recorded after the short delay was of shorter duration than that obtained after the long interval. The amplitude of the spike potential, which in drone retinula cells occurs at the beginning of the receptor potential, as well as that of the receptor potential itself were the same for both delays and for the control.

EFFECTS OF CALCIUM IONS Changes in the shape of the receptor potential, similar to those resulting from the aftereffects of illumination can be induced in dark-adapted preparations by increasing the intracellular concentration of calcium. In the experiment illustrated in Fig. 7, the retina was stimulated with flashes of constant duration and intensity at a rate sufficiently low to permit the cell to adapt almost completely to darkness during the interval between two flashes. A solution of CaEGTA with a calculated free calcium concentration of 3.3×10^{-6} mol/liter was injected into the cell by pressure and the injection was monitored by passing short negative current pulses through the intracellular micropipette. Injection of CaEGTA caused a shortening of the duration of the receptor potential and a reduction of its amplitude (Fig. 7*b*). Complete recovery of the shape of the receptor potential occurred 2–6 min after the heating current had been turned off (Fig. 7*c*). This recovery time is longer than that usually observed after exposure to a background light. This difference might be explained by the fact that CaEGTA continued to be forced into the cell during the cooling of the pressure device (see Methods).

Similar changes in the shape of the receptor potential were also observed when a solution of CaCl_2 containing no EGTA was injected into retinula cells. Micropipettes containing CaCl_2 alone, however, proved less satisfactory for experimental purposes than those containing CaEGTA; their resistance very often became extremely high in the course of injection thus making recording of the receptor potential impossible.

In contrast to the effect of an increase in the intracellular concentration of calcium, a decrease in intracellular calcium induced by the injection of EGTA alone caused changes similar to those of dark adaptation. This is illustrated in Fig. 8. In this experiment the preparation was stimulated regularly with a flash of constant intensity and duration. Intracellular injection of EGTA was found to cause a transient increase in the duration of the receptor potential. Complete recovery of the receptor potential occurred 2 min after the heating current was discontinued.

A similar increase in duration of the receptor potential could also be obtained either by increasing the interval between two test flashes (Fig. 9), or by reducing calcium concentration in the bathing medium in which the preparation was stimulated with test flashes at a constant rate (Fig. 10). Thus, the lowering of either the extracellular or the intracellular calcium concentration was found to cause changes in the shape of the receptor potential closely resembling those observed in the course of dark adaptation. Conversely, an increase in either the intracellular or the extracellular calcium concentration caused changes similar to those of light adaptation. Therefore, calcium appears to be involved, not only in the changes of the shape of responses of retinula cells to test flashes superposed on a background light described earlier in this paper, but also in the aftereffects observed after the background light has been extinguished.

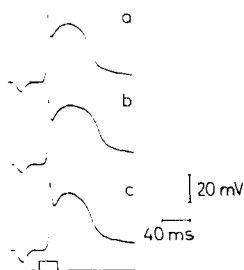


FIGURE 8

FIGURE 8. Effects of intracellular injection of EGTA on the response to a short flash. The preparation was stimulated regularly with flashes of 30-ms duration at a rate of one flash per 10 s. EGTA was injected by pressure (heating current 2.2 A; duration 6 s). *a* shows the response to the flash recorded before the injection of EGTA, *b* the response recorded 20 s, and *c* the response recorded 2 min after the end of the heating current.

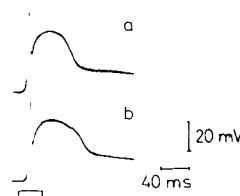


FIGURE 9

FIGURE 9. Effect of the rate of stimulation on the response to a short test flash. The intensity of the flash was -2.4 and its duration 30 ms. In *a* the flash was applied at a rate of one flash per 10 s, and in *b* at a rate of one flash per 30 s.

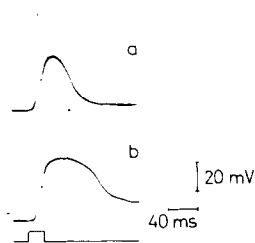


FIGURE 10

FIGURE 10. Effects of a decrease in calcium concentration in the bathing medium on the response to a short flash. In *a* the calcium concentration was 1.8 mmol/liter and in *b* 0.1 mmol/liter. Flash of 20-ms duration, applied at a rate of one per 10 s.

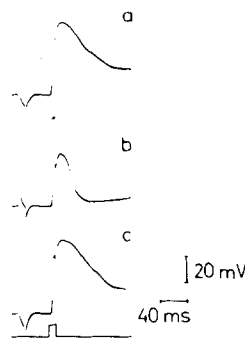


FIGURE 11

FIGURE 11. Effects of intracellular injection of NaCl on the response to a short flash. NaCl was injected by pressure (heating current 2 A and 20-s duration). *a* shows the response before the injection; *b* and *c* 50 and 460 s, respectively, after the heating current was discontinued. Flash of 10-ms duration, applied at a rate of one per 10 s.

EFFECTS OF SODIUM IONS If the increase in intracellular calcium is the mechanism by which exposure of a retinula cell to an adapting light affects the receptor potential, the question naturally arises as to how illumination can lead to this increase. One explanation would be that there is an influx of calcium ions into the retinula cell during the receptor potential. On the other hand, sodium ions are known to be the major carriers of the receptor current (Fulpius and Baumann, 1969), and entry of sodium could cause, secondarily, an increase in the intracellular calcium concentration. This possibility is suggested by the ob-

ervation that intracellular injection of sodium can affect the shape of the receptor potential in much the same way as does either an intracellular injection of calcium or exposure to light.

Injection of sodium ions by pressure reduced the duration and amplitude of the receptor potential (Fig. 11). Similar changes in the shape of the receptor potential were also observed when the sodium ions were injected by iontophoresis. Recovery of the receptor potential to its original value occurred about 3 min after injection of sodium by iontophoresis. This time was approximately the same as that required for recovery after exposure to light. When the sodium was injected by pressure, however, recovery was significantly slower, due to the fact already mentioned that sodium continued to be forced into the cell during cooling of the pressure device.

Intracellular injection of sodium did not affect the size of the spike potential. However, it usually caused a slight and transient increase in the membrane potential of the retinula cell. A transient hyperpolarization was also generally observed after exposure of a retinula cell to a steady adapting light. This hyperpolarization is probably caused by an activation of the electrogenic sodium pump of the retinula cell by the increase in the intracellular sodium concentration.

INJECTION OF OTHER CATIONS In order to determine whether the effects of sodium and calcium on the shape of the receptor potential described above were due to the increase in intracellular concentration of these cations or to some unspecific effect of intracellular injection, three other ions (K, Li, Mg) were also injected into retinula cells. Fig. 12 shows that injection of potassium ions by pressure had no effect on the shape of the receptor potential.

When potassium was injected iontophoretically, however, the response recorded immediately after discontinuing the injecting current was found to be prolonged in duration and increased in amplitude (Fig. 13). These effects were generally accompanied by a slight depolarization of the retinula cell. The modifications observed in both the receptor potential and the membrane potential disappeared rapidly (in less than 30 s) after the injecting current was discontinued and were probably due to the depolarization of the cell which occurred during iontophoresis rather than to an increase in intracellular potassium concentration. This depolarization could have caused a change in the concentration gradient of chloride ions across the cell membrane. It has been shown, in fact, that retinula cells of the honey bee drone are permeable to chloride ions and that the distribution of these ions across the membrane is determined by the membrane potential (Baumann and Hadjilazaro, 1972). Hence, the decrease in membrane potential during iontophoresis of potassium could cause an influx of chloride ions into the cell and a resulting reduction in their concentration gradient. This would lead to a reduction in the influx of chloride ions during the receptor potential, which, as shown by Baumann and Hadjilazaro (1972) in an experiment in which chloride in the bathing medium was replaced by an impermeant anion, causes an increase in the duration of the receptor potential similar to that observed after iontophoretic injection of potassium.

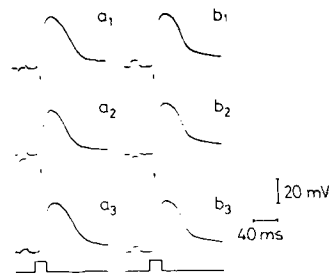


FIGURE 12

FIGURE 12. Comparison of the effects on the receptor potential of injection by pressure of NaCl, 2 M (*a*) and KCl 3 M (*b*) into two different cells. *a*₁ and *b*₁ were recorded before the injection; *a*₂ and *b*₂ shortly after the heating current was discontinued (heating current: 1 A and 15-s duration in *a* and 1.2 A and 20-s duration in *b*). It can be seen in both *a*₂ and *b*₂ that the balance of the bridge is upset indicating that the solutions are being injected into the cells. *a*₃ shows the response recorded 6 min after the maximal change in the shape of the receptor potential had occurred, *b*₃ shows the response recorded 1 min after the maximal change in the bridge balance. Flash of 20-ms duration, applied at a rate of one per 10 s.

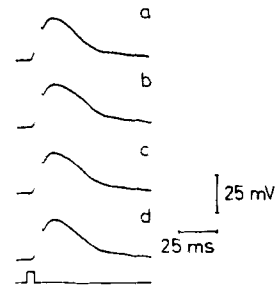


FIGURE 13

FIGURE 13. Effects of intracellular injection of potassium by iontophoresis. *a* shows the response recorded before the injection; *b* shows the response recorded immediately after the end of the iontophoretic current; *c* 10 s and *d* 40 s later. A 30-s current of 50 nA was passed through the micropipette containing KCl 3 M. Flash of 5-ms duration, applied at a rate of one per 10 s.

The effect of membrane depolarization during iontophoresis on the duration of the receptor potential was also observed when sodium was injected into retinula cells. In this case, however, it was partially masked by the effect of sodium itself, which is to reduce the duration of the receptor potential. Thus, the duration of the receptor potential recorded immediately after the iontophoretic current was discontinued, although shorter than that of the control, was consistently longer than that of a response recorded 30 s later.

Lithium and magnesium were also injected into retinula cells and neither was found to affect the shape of the receptor potential in the same way as sodium or calcium ions. Some changes in the shape of the receptor potential were observed, but these may have been due, as was the case for potassium, to the method of injection rather than to the ion itself.

DISCUSSION

When the retina of the honey bee drone is exposed to light, several changes are known to occur, such as migration of accessory pigment granules (Kolb and Autrum, 1972), shrinking of the subrhabdomeric cisternae (Baumann et al., 1967), changes in the spectral absorbance of the visual pigment similar to those described in a moth by Hamdorf et al. (1973) (Muri and Baumann, unpublished), as well as changes in the intracellular concentration of certain ions. Some of these changes have been considered responsible for the decrease in sensitivity to light that is characteristic of light adaptation. In the present study, it was

found that the intracellular injection of calcium and sodium, unlike that of the other cations tested, caused changes in the shape of the receptor potential closely resembling those observed in retinula cells immediately after exposure to a background light. This finding suggests that the increase in the intracellular concentration of both calcium and sodium is responsible for light adaptation, at least under the experimental conditions of the present study (illumination of the ommatidia with white light [Xenon arc] applied perpendicularly to their longitudinal axis).

The essential part of the mechanism leading to light adaptation seems to be an increase in intracellular calcium concentration. This is suggested by the fact that EGTA, which has no affinity for sodium and which does not reduce the influx of sodium during the receptor potential, causes changes on intracellular injection resembling those of dark adaptation in a light-adapted cell and prevents light adaptation in a dark-adapted cell. It may be assumed that this effect of EGTA was due to its calcium binding capacity rather than to some unspecific effect of the EGTA molecule or to the injection procedure since it was found that EGTA increased the duration of the receptor potential while CaEGTA reduced it. A further indication that EGTA acted by lowering the intracellular calcium concentration is the fact that its injection into the cell caused the same changes in the shape of the receptor potential as did the lowering of the calcium concentration in the bathing medium.

It has been shown that neutralization of light adaptation by EGTA could be prevented by increasing the intensity or the frequency of the stimulation. This can be explained if it is assumed that the increase in free calcium concentration in the cell cytoplasm during the receptor potential is related to the intensity of the light stimulus, and that the mechanisms thought to control the free calcium concentration in the cytoplasm are rate limited. Reducing the intensity of the stimulation or increasing the interval between two stimuli might allow these mechanisms to restore the resting calcium concentration, lowering the $[\text{Ca-EGTA}]/[\text{EGTA}]$ ratio, thus enabling EGTA to bind the calcium ions that accumulate during the light response. Conversely an increase in the light intensity or in the frequency of the stimulation would tend to reduce the EGTA concentration, while increasing that of CaEGTA, so that the EGTA concentration might be insufficient to prevent the calcium ions from participating in the mechanism of light adaptation.

Direct evidence that illumination actually induces an increase in the intracellular calcium concentration has recently been provided by Brown and Blinks (1974) who used aequorin to detect changes in intracellular calcium concentration in the photoreceptor cells of the *Limulus* ventral eye. In this preparation, voltage clamp experiments have shown that illumination with a background light gives rise to a strong initial current which decreases to a smaller plateau (Millecchia and Mauro, 1969). Lisman and Brown (1975 *b*) found that this decline was due to an increase in the intracellular free calcium concentration which caused a decrease in the conductance change per incident photon; this, they suggested, could account for the decrease in sensitivity of the photoreceptor cells (Lisman and Brown, 1975 *a*). These results agree with those described here in which it has

been shown that, in the retinula cells of the honey bee drone, an increase in the intracellular calcium concentration leads to a drop in the receptor potential from the transient to the plateau and that this drop accurately reflects the decrease in sensitivity to light.

Although the calcium seems to be the determining factor leading to light adaptation, sodium might also play a role since intracellular injection of sodium ions were also found to affect the shape of the receptor potential in the same way as did light adaptation. The changes in the shape of the receptor potential induced by the intracellular injection of sodium were probably caused by increases in intracellular sodium too small to cause a reduction in the concentration gradient of sodium ions across the membrane and a consequent decrease in the influx of sodium during the receptor potential. This is suggested by the finding that the amplitude of the spike potential which seems to originate in the retinula cell (Shaw, 1969) and which has been shown to be dependent on the sodium gradient (Baumann and Fulpius, 1968) was not affected by the intracellular injection of sodium.

The effect of an intracellular injection of sodium on the shape of the receptor potential could be explained by an increase in intracellular free calcium triggered by a small increase in intracellular sodium. This increase in intracellular free calcium could be caused either by liberation of calcium ions from intracellular sites such as mitochondria (Carafoli et al., 1974) or subrhabdomic cisternae (Perrelet, unpublished), or by sodium-calcium exchange through the membrane of the retinula cell (Lisman and Brown, 1972; Blaustein, 1974). If sodium can actually cause an increase in calcium concentration in retinula cells, then it could be the light-evoked influx of sodium ions into the cell that triggers the mechanism leading to light adaptation. It has been found (Baumann and Mauro, 1973, 1974) that in the honey bee drone, several experimental conditions known to inhibit the function of the sodium pump and thereby to lead to an increase in intracellular sodium cause a decrease in sensitivity to light by reducing the light-evoked conductance change.

It is therefore possible that, in the honey bee drone, the loss in sensitivity observed during light adaptation is the result of an increase in intracellular sodium which leads to an increase in intracellular calcium. This, in turn, causes a reduction in the light-induced conductance changes. Experimental evidence available at present, however, is insufficient to exclude the possibility that the increase in intracellular calcium is the result of an influx of calcium ions into the retinula cell caused by a direct effect of light on calcium permeability (Brown et al., 1970), or is due to an intracellular release of calcium that is not mediated by the increase in the intracellular sodium concentration.

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REFERENCES

- BADER, C. R., D. BERTRAND, G. BERTRAND, and A. PERRELET. 1974. A pressure device for intracellular injection. *Experientia (Basel)*. **30**:1366-1367.
- BAUMANN, F. 1968. Slow and spike potentials recorded from retinula cells of the honey bee drone in response to light. *J. Gen. Physiol.* **52**:855-875.
- BAUMANN, F. 1972. Influence of light adaptation and intracellular injection of sodium on the receptor potential of drone retinula cells. *J. Physiol. (Lond.)*. **226**:114-115P.
- BAUMANN, F., and B. FULPIUS. 1968. Effect of sodium and calcium ions on slow and spike potentials in single photoreceptor cells. *Proc. Int. Union Physiol. Sci.* **7**:33.
- BAUMANN, F., and B. HADJILAZARO. 1972. A depolarizing aftereffect of intense light in the drone visual receptor. *Vision Res.* **12**:17-31.
- BAUMANN, F., and A. MAURO. 1973. Effect of hypoxia on the change in membrane conductance evoked by illumination in arthropod photoreceptors. *Nat. New Biol.* **244**:146-148.
- BAUMANN, F., and A. MAURO. 1974. Effets de l'hypoxie et de l'ouabaïne sur le potentiel récepteur de la cellule rétinienne du faux-bourdon. *J. Physiol. (Paris)*. **69**:187A.
- BAUMANN, F., A. PERRELET, and B. FULPIUS. 1967. Etude fonctionnelle et morphologique de la cellule rétinienne du faux-bourdon au cours de l'adaptation à la lumière et à l'obscurité. *Helv. Physiol. Pharmacol. Acta.* **25**:CR163.
- BLAUSTEIN, M. P. 1974. The interrelationship between sodium and calcium fluxes across cell membranes. *Rev. Physiol. Biochem. Pharmacol.* **70**:33-82.
- BROWN, H. M., S. HAGIWARA, H. KOIKE, and R. M. MEECH. 1970. Membrane properties of a barnacle photoreceptor examined by the voltage clamp technique. *J. Physiol. (Lond.)*. **208**:385-413.
- BROWN, J. E., and J. R. BLINKS. 1974. Changes in intracellular free calcium concentration during illumination of invertebrate photoreceptors. Detection with aequorin. *J. Gen. Physiol.* **64**:643-665.
- CARAFOLI, E., R. TIOZZO, G. LUGLI, F. CROVETTI, and C. KRATZING. 1974. The release of calcium from heart mitochondria by sodium. *J. Mol. Cell Cardiol.* **6**:361-371.
- DOWLING, J. E., and H. RIPPS. 1972. Adaptation in skate photoreceptors. *J. Gen. Physiol.* **60**:698-718.
- FEIN, A., and R. D. DE VOE. 1973. Adaptation in the ventral eye of *Limulus* is functionally independent of the photochemical cycle, membrane potential, and membrane resistance. *J. Gen. Physiol.* **61**:273-289.
- FULPIUS, B., and F. BAUMANN. 1969. Effect of sodium, potassium, and calcium ions on slow and spike potentials in single photoreceptor cells. *J. Gen. Physiol.* **53**:541-561.
- GOLDSMITH, T. H. 1963. The course of light and dark adaptation in the compound eye of the drone honey bee. *Comp. Biochem. Physiol.* **10**:227-237.
- HAMDORF, K., G. HÖGLUND, and H. LANGER. 1973. Photoregeneration of visual pigment in a moth. A microphotometric study. *J. Comp. Physiol.* **86**:247-263.
- KOLB, G., and H. AUTRUM. 1972. Die Feinstruktur im Auge der Biene bei Hell- und Dunkeladaptation. *J. Comp. Physiol.* **77**:113-125.
- LISMAN, J. E., and J. E. BROWN. 1972. The effects of intracellular injection of calcium and sodium ions on the light response of *Limulus* ventral photoreceptors. *J. Gen. Physiol.* **59**:701-719.
- LISMAN, J. E., and J. E. BROWN. 1975 a. Light-induced changes of sensitivity in *Limulus* ventral photoreceptors. *J. Gen. Physiol.* **66**:473-488.

- LISMAN, J. E., and J. E. BROWN. 1975 *b*. Effects of intracellular injection of calcium buffers on light adaptation in *Limulus* ventral photoreceptors. *J. Gen. Physiol.* **66**:489-506.
- MILLECCHIA, R., and A. MAURO. 1969. The ventral photoreceptor cells of *Limulus*. III. A voltage clamp study. *J. Gen. Physiol.* **54**:331-351.
- NAKA, K., and K. KISHIDA. 1966. Retinal action potentials during light and dark adaptation. *In* Functional Organization of the Compound Eye. C. G. Bernhard, editor. Pergamon Press, Oxford. 251-266.
- SHAW, S. R. 1969. Interreceptor coupling in ommatidia of drone honeybee and locust compound eyes. *Vision Res.* **9**:999-1029.