Effects of Sodium, Potassium, and Calcium Ions on Slow and Spike Potentials in Single Photoreceptor Cells

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ABSTRACT The influence of changes in the ionic composition of the bathing medium on responses of the retinula cell of the honeybee drone to light was examined by means of intracellular microelectrodes. The resting potential of the cell was influenced mainly by the concentration of K. The peak of the receptor potential (the transient), which in a normal solution and with strong light approaches zero membrane potential, overshot this level in a K-rich solution. An increase in the concentration of K also raised the level of the steady-state phase of the receptor potential (the plateau). The amplitude of the receptor potential was decreased and the spike potential rapidly abolished when Na was replaced by either sucrose, choline, or Tris. In a Ca-free solution the amplitude of the response and especially that of the plateau, was increased. An increase in Ca had the opposite effects. All these changes were reversible. An attempt was made to interpret the receptor and spike potentials in terms of passive movements of Na and K across the membrane of the retinula cell. The major difficulty encountered was to find an explanation for the persistence of an appreciable fraction of the transient and the plateau in preparations kept up to 12 hr in a solution in which all the Na had been replaced by choline, Tris, or sucrose.

INTRODUCTION

The response to light of the retinula cell of the honeybee drone, as of several other invertebrates, consists of a slow depolarization, the receptor potential (Naka and Eguchi, 1962). It is generally believed (following the work of Katz, 1950; Diamond et al. 1958; and Fuortes, 1959) that receptor potentials are due to a modification in membrane permeability to one or more ions, which pass through the membrane and, moving down their electrochemical gradients, change its potential. On the basis of this hypothesis, several investigators, using intracellular microelectrodes, have studied the influence of the ions in the extracellular fluid on the receptor potential of retinula cells

(Kikuchi et al., 1962; Eguchi, 1965; Millecchia et al., 1966; Smith, 1966). The changes observed suggest that the current generating the receptor potential is mainly due to sodium.

Naka and Eguchi (1962) found in the honeybee drone a spike potential superimposed on the initial phase of the receptor potential; they thought it might originate in the axon of the retinula cell. Baumann (1968) observed that this spike could be induced by electric stimulation of the retinula cell, and that it was abolished in a solution containing tetrodotoxin. He concluded that the retinula cell membrane of the drone has two typical properties, one responsible for producing the receptor potential and the other for the spike. The aim of the present study was to investigate and compare the effect of changes in the concentration of potassium, sodium, and calcium on the receptor and spike potentials. The results confirm the hypothesis that the spike potential resembles that of nerve fiber. Sodium ions appear to contribute largely to the generation of the receptor potential, but in the drone, their influx into the cell does not seem to be the only process implicated. Two brief preliminary accounts of this work have already appeared (Fulpius and Baumann, 1966, 1967).

METHODS

The Preparation The head of the honeybee drone (*Apis mellifica* L.) was isolated and divided into two parts by a section across the two eyes perpendicular to the cornea. The larger, anterior part was placed in a small plexiglass chamber holding 0.6 ml, which was continuously perfused at the rate of approximately 3.6 ml/min. The experiments were performed at a room temperature varying between 19° and 23°C. The solution in the chamber could be changed in about 1 min. The effects of a new solution were observed for periods of perfusion lasting from several minutes to several hours.

Solutions All solutions were freshly prepared. Whenever possible the concentrations were adjusted to give a freezing point depression corresponding to 370 ± 10 milliosmols, measured in a Fiske osmometer. The pH was adjusted to 7.3 ± 0.1. The composition of the physiological solution, the same as that used by Baumann (1968), is shown in Table I, line A. The composition of the solutions in which sodium was replaced is shown in lines B to E; the solutions in which [K] was modified are shown in F to H, and changes in [Ca] in I and J. Partial substitutions were obtained by appropriate mixing with the physiological solution.

Recording Intracellular potentials were recorded by means of glass electrodes filled with 3 M KCl solution. The DC resistance of the electrodes was between 15 and 30 MΩ. The electronic equipment consisted of a Bak wide band electrometer (Bak, 1958) with negative capacitance feedback and an oscilloscope (Tektronix 565). In some experiments the two channels of the oscilloscope were used to display the potential changes simultaneously with a slow (1 cm/200 msec) and a fast (1 cm/5 msec) sweep speed.

Stimulation The light source was a tungsten filament or a xenon arc (XBO,

150 w). The beam passed through a heat-absorbing filter and was focused on the plane of a diaphragm. It was then projected through a collimating lens and a microscope objective lens on to the preparation. The illuminated surface, about 1.0 mm in diameter, included the whole length of an ommatidium. Stimulus intensity could be varied by calibrated neutral filters and its duration controlled by an electromagnetic shutter at the diaphragm. The unattenuated light stimulus was recorded by a photoelectric cell (RCA 929). The intensity of the light stimulus is expressed on a logarithmic scale where 0 corresponds to the unattenuated light. The positions of the light beam and the electrode on the preparation were controlled by viewing through a stereomicroscope.

Measurements The amplitudes (V) of the different components of the retinula cell response to light were measured in relation to the resting potential of the cell at the beginning of the experiment. When V could not be determined, in experiments in which changing the solution had modified the junction potential between the bathing medium and the neutral electrode, the amplitudes have been expressed as the difference between each component of the response and the potential recorded immediately before stimulation. In some experiments the resting potential relative to 0 potential was determined by withdrawing the electrode from the cell.

TABLE I

COMPOSITIONS OF THE SOLUTIONS

	$Choline^+$	K^+	Li^+	Na^+	$TrisH^+$	Ca^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	HPO_4^{2-}	Glucose	Saccharose
					mg ion/liter						*mmole/liter*	
A	—	3.2	—	203.5	—	1.8	206.5	—	0.2	1.8	5.6	—
B	—	3.2	—	3.8	—	1.8	6.8	—	0.2	1.8	5.6	324.0
C	199.7	3.2	—	3.8	—	1.8	206.5	—	0.2	1.8	5.6	—
D	—	3.2	—	—	200.0	1.8	206.8	—	—	—	5.6	—
E	—	3.2	199.7	—	10.0	1.8	216.5	—	—	—	5.6	—
F	—	—	—	203.5	—	1.8	203.3	—	0.2	1.8	5.6	—
G	—	32.0	—	203.5	—	1.8	235.3	—	0.2	1.8	5.6	—
H	—	64.0	—	203.5	—	1.8	267.3	—	0.2	1.8	5.6	—
I	—	3.2	—	203.5	—	—	202.9	—	0.2	1.8	5.6	—
J	—	3.2	—	206.8	—	18.0	238.9	7.1	—	—	5.6	—

A is the physiological solution. B to J are solutions of modified ionic composition.

RESULTS

Light Response of the Retinula Cell in the Physiological Solution The response of the retinula cell to weak light, measured by intracellular microelectrodes, consists of a depolarization maintained throughout the duration of illumination (Fig. 1, −6.0). Discrete irregular waves similar to those observed in *Limulus* (Yeandle, 1958), the grasshopper (Scholes, 1964), and the fly (Kirschfeld, 1965) are superimposed on the depolarization induced by the light. Above a certain intensity of stimulus (−4.8), the response shows an initial peak depolarization (the transient) followed by a smaller sustained depolar-

ization (the plateau). At higher intensities, the transient becomes pronounced and is followed by a slow, strongly damped oscillation. In the experiment illustrated in Fig. 1, a spike appears at an intensity of −3.6. Fig. 2 shows the amplitudes of spike, transient, and plateau plotted against the log of the light intensity. The amplitude of the transient increases along a "sigmoid" curve and reaches a constant value around 0 potential. Thus stimulation with high intensities abolishes temporarily the difference of potential between the exterior and interior of the cell at the peak of the slow potential. The amplitude of the plateau also reaches a constant value, but unlike the transient, this always remains below 0 potential. In the example shown in Fig. 2 it is

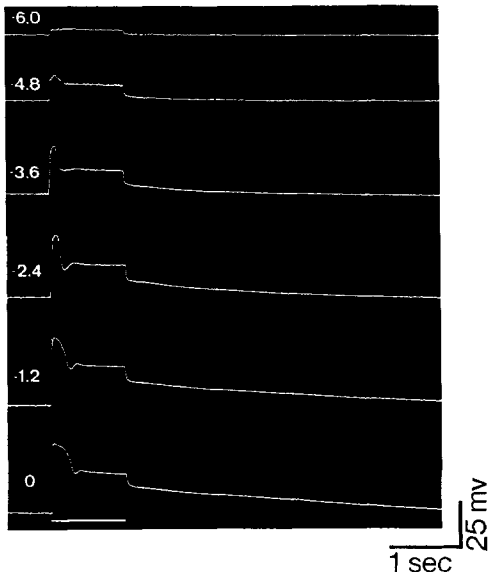

FIGURE 1. Responses to light of increasing intensity and of 1 sec duration. Light intensity, expressed in log units, is indicated at the left of each response. The three components of the response, spike, transient, and plateau, are best seen with intensity −3.6.

about −20 mv. In many cells, contrary to both transient and plateau, the spike overshoots 0 potential in response to a strong light stimulus.

Effect of Potassium In the drone extracellular [K] has a marked influence on the resting potential of the retinula cell. An increase from 3.2 to 32.0 mg ion/liter (solution G, Table I) resulted in an average depolarization of 15.5 mv (five cells: extreme values, 8 mv and 25 mv), and an increase from 3.2 to 64.0 mg ion/liter (solution H, Table I) in a depolarization of 32.0 mv (eight cells: extreme values, 23 mv and 49 mv). It can be noticed that these values are smaller than those expected for a 10-fold increase in [K] and for a membrane solely permeable to potassium (58 mv for a 10-fold change). This deviation from the Nernst equation may be due to a slight permeability of the nonilluminated retinula cell to sodium. It may also be due in part to a shunt of the membrane caused by the penetration of the cell by the microelectrode.

In addition to its effect on the membrane potential, the increase in [K] modified the form and amplitude of the light response (Fig. 3). The amplitudes of both transient and plateau were reduced. Total depolarization however, i.e., change in resting potential plus response, was greater than that induced by illumination in the physiological medium (Fig. 4). At high light

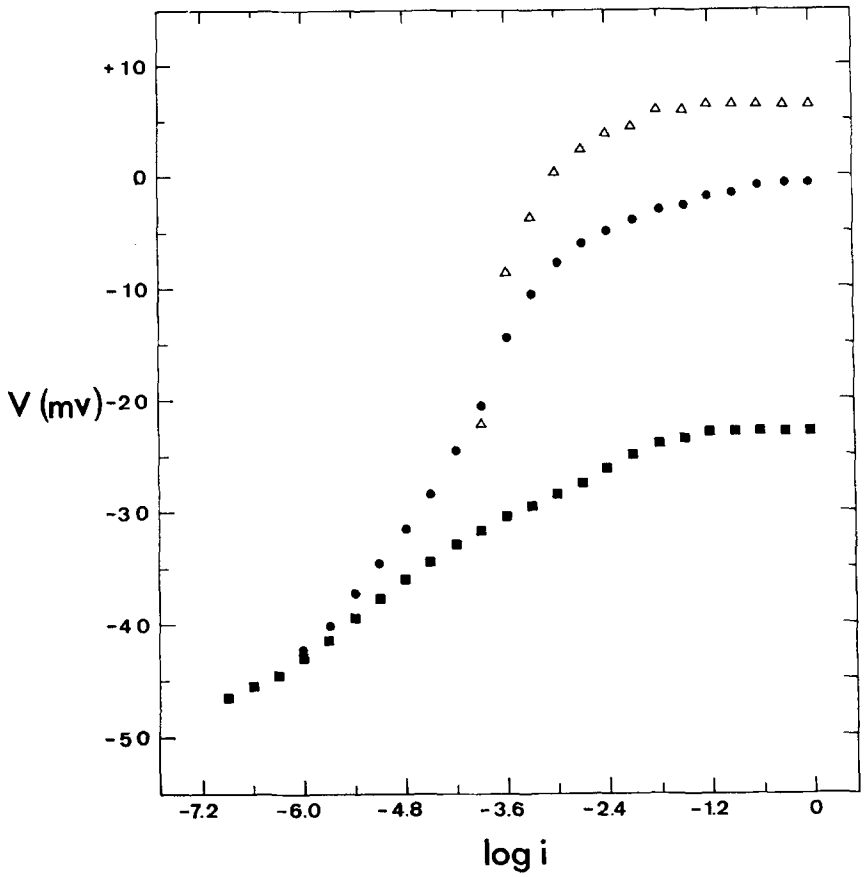

FIGURE 2. Effect of light intensity on the membrane potential of a retinula cell measured at the maximum of the transient (filled circles), at the end of the plateau (filled squares), and at the top of the spike (open triangles). On the ordinate, membrane potential, V; on the abscissa, log of light intensity, i.

intensities the transient overshot 0 potential. This was observed in all experiments using a potassium-rich solution. The spike showed three types of modification. In some cells it was abolished (Fig. 5 a), probably owing to a relatively large depolarization of the membrane and consequent sodium inactivation. In others the amplitude of the spike relative to the resting potential in the normal medium was unchanged (Fig. 5 b). It will be ob-

served that in the experiment illustrated by Fig. 5 b the depolarization caused by the increase in [K] was less than that illustrated in Fig. 5 a. In some cells where no spike occurred in the normal medium the increase in [K] caused it to appear (Fig. 5 c). The changes in resting potential, spike, transient, and plateau resulting from an increase in external [K] were completely reversible (Fig. 6).

Effect of Sodium The role of this ion in generating the retinula cell potential in the drone was investigated by total or partial replacement of

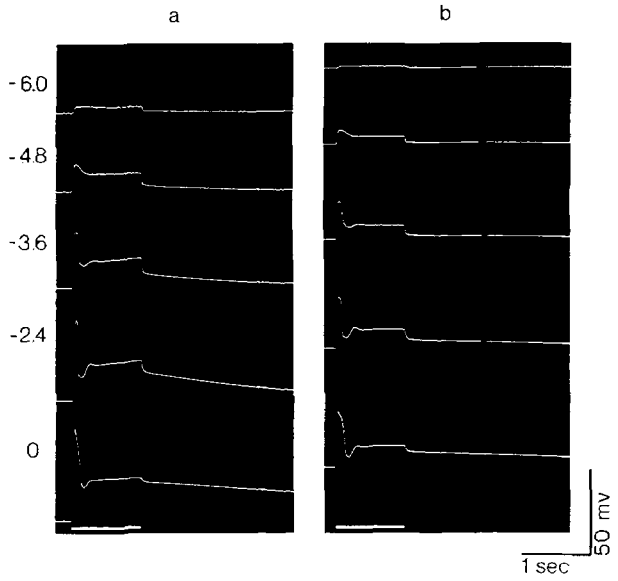

FIGURE 3 Effects of an increase in [KCl] on responses to light. In a, responses to light of increasing intensity recorded in the normal solution (KCl = 3.2 mM); in b, the responses to the same light intensities recorded in the same cell but in a solution containing 64.0 mM of KCl. The depolarizing action of K appears in this figure as an upward shift of the responses in b relative to a. Light intensity expressed in log units is indicated at the left of the figure.

the sodium in the physiological medium by the following substitutes, choline chloride, tris(hydroxymethyl)aminomethane, sucrose, and lithium chloride.

Replacement of Na by TrisH or Choline These two substitutes had very similar effects on the retinula cell response to light, although the TrisH solution was entirely free from Na (solution D, Table I) while 3.6 mg ion/liter of Na remained in the choline solution through the use of the usual buffer (solution C, Table I). None of the effects observed when choline was substituted for Na was modified by the addition of atropine (10^{-4} g/liter), and a cholinergic activity of this substance can therefore, in this case, be dismissed. In the absence of sodium, the amplitude of the response to a weak light stimu-

lus (−6.0) was considerably diminished, as were the discrete waves (Fig. 7). In response to stronger stimuli, the amplitude of the plateau was reduced more markedly than that of the transient; the duration of the transient was reduced, and the resting potential increased by about 5 mv. This slight

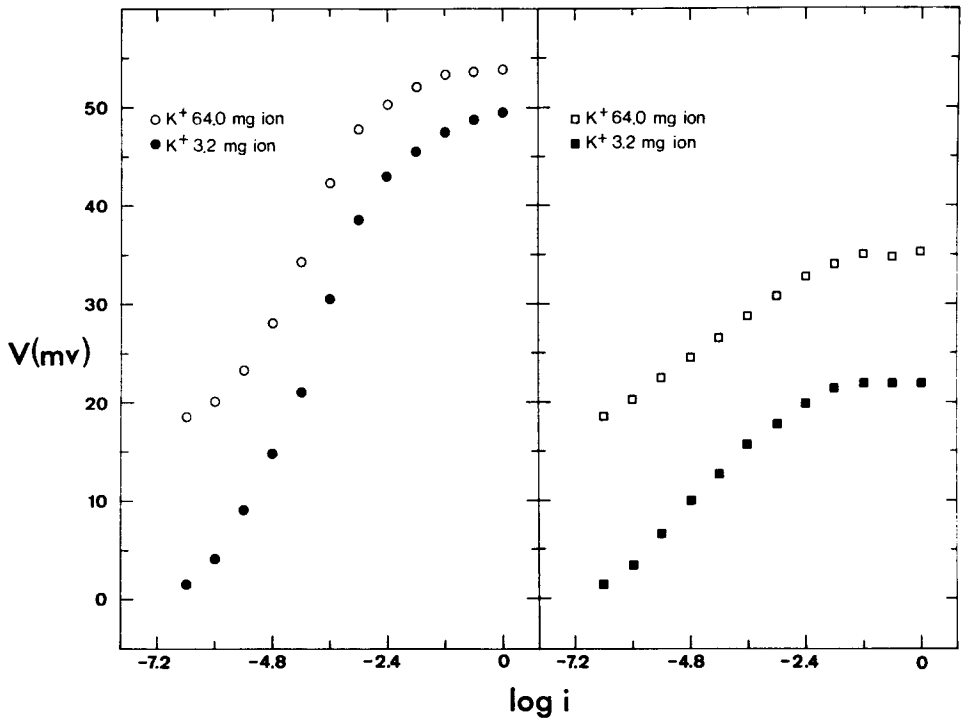

FIGURE 4. Influence of [KCl] on the relation between voltage and light intensity. The amplitudes, V, of the transient (open and filled circles) and plateau (open and filled squares) were measured relative to the resting potential determined at the beginning of the experiment. The responses obtained in the solution containing 3.2 mM KCl (filled circles and squares) were determined before those obtained in the solution containing 64.0 mM KCl (open circles and squares).

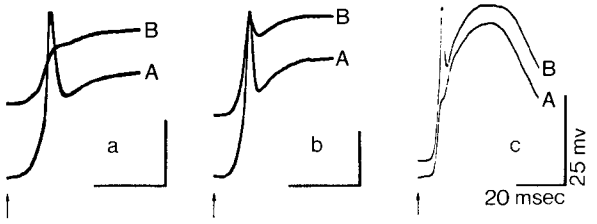

FIGURE 5. Influence of an increase in [KCl] from 3.2 to 32.0 mM on spike potentials recorded in three different cells, a, b, and c. Trace A recorded before and trace B during application of a solution of increased [KCl] are photographically superposed. The onset of light stimulation is indicated by an arrow. Stimulation duration was 1 sec in a and b, 20 msec in c.

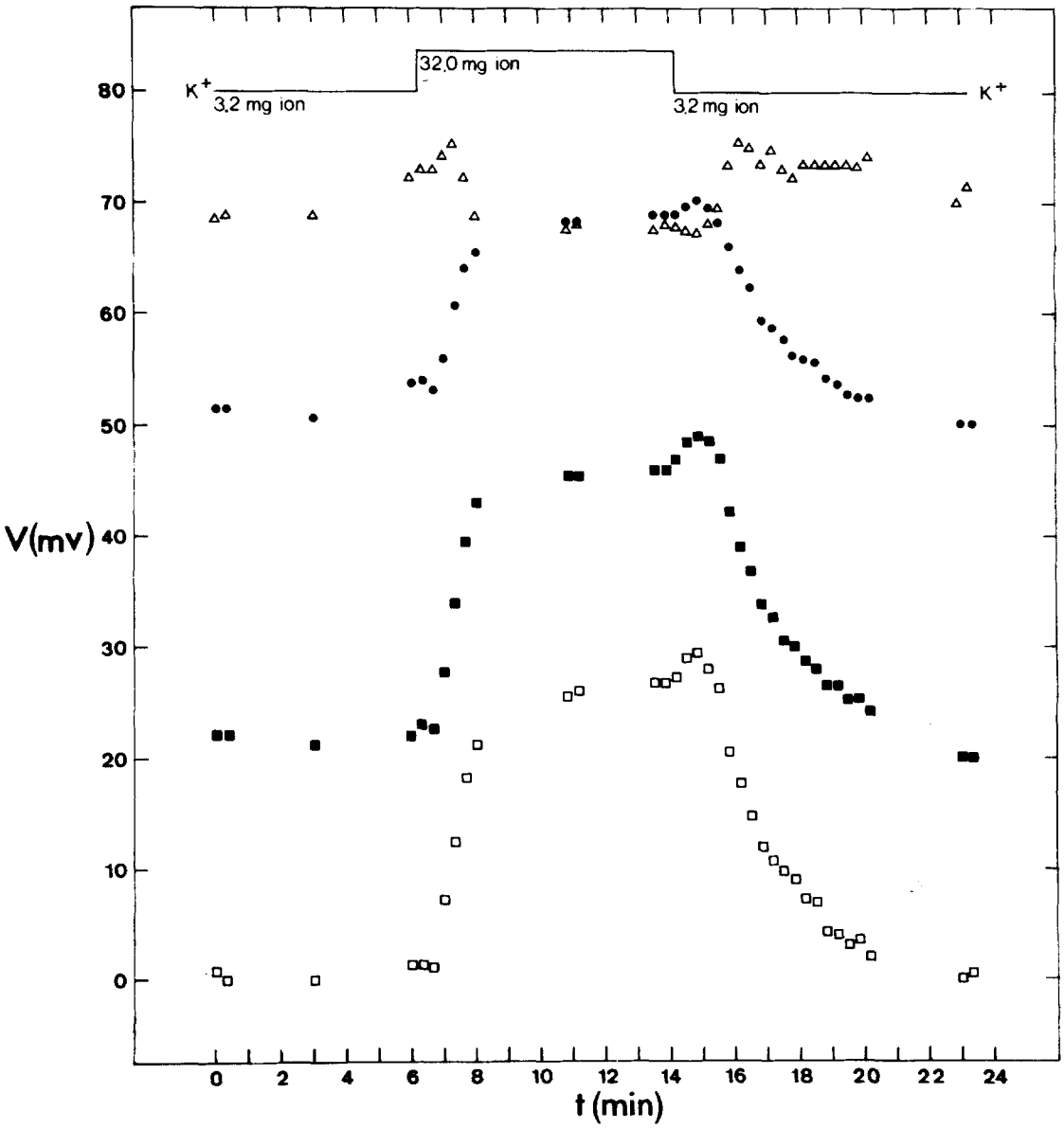

FIGURE 6. Effects of a 10-fold increase in [KCl] on the resting potential and on the amplitude of the response to intense light of 1 sec duration. The preparation was stimulated once every 10 sec. The amplitudes, V, of the transient (filled circles), plateau (filled squares), and spike potentials (open triangles) were measured relative to the resting potential (open squares) determined at the beginning of the experiment. The small hump visible when [KCl] is reduced is probably due to a change in the junctional potential between the solution and the indifferent electrode.

hyperpolarization suggests that when not illuminated the membrane of the retinula cell is slightly permeable to Na. The shape of the relation between voltage and light intensity was not affected by the replacement of Na but all the amplitudes were reduced (Fig. 8). In a sodium-free solution not even the strongest light was capable of abolishing the difference of potential across the membrane, contrary to what was observed in the normal medium. The rate of depolarization of the membrane was reduced (Fig. 9 a). In the experiment illustrated in Fig. 9 the place of the spike on the rising phase of the slow potential is marked by a slight residuary hump; this disappeared when

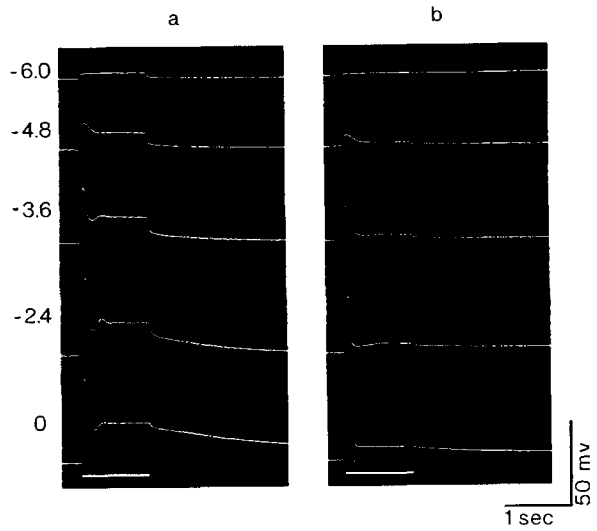

FIGURE 7. Effects of replacing Na by TrisH. In a, responses to light of increasing intensity recorded in the normal solution (NaCl = 203.5 mM); in b, the responses to the same light intensities recorded in a sodium-free solution. Stimulus duration was 1 sec. Light intensity expressed in log units is indicated at the left of the figure.

the preparation was left for a longer time in the sodium-free solution. Fig. 9 b shows that the disappearance of the spike was not due to the diminished amplitude of the receptor potential or to a reduced rate of depolarization. The changes in response to a strong light stimulus observed during a short period of sodium substitution (less than 10 min) are illustrated in Fig. 10. It is seen that all the changes were reversible and that the recovery of the amplitudes in the physiological solution was faster than their diminution in the sodium-free solution. The recovery seems to follow a monotonic function. This was not the case when sodium was substituted for a longer period. As shown in Fig. 11, in this condition the amplitude of the plateau temporarily reached values higher than any observed before the sodium substitution. After a prolonged substitution, the spike appeared late; its amplitude in-

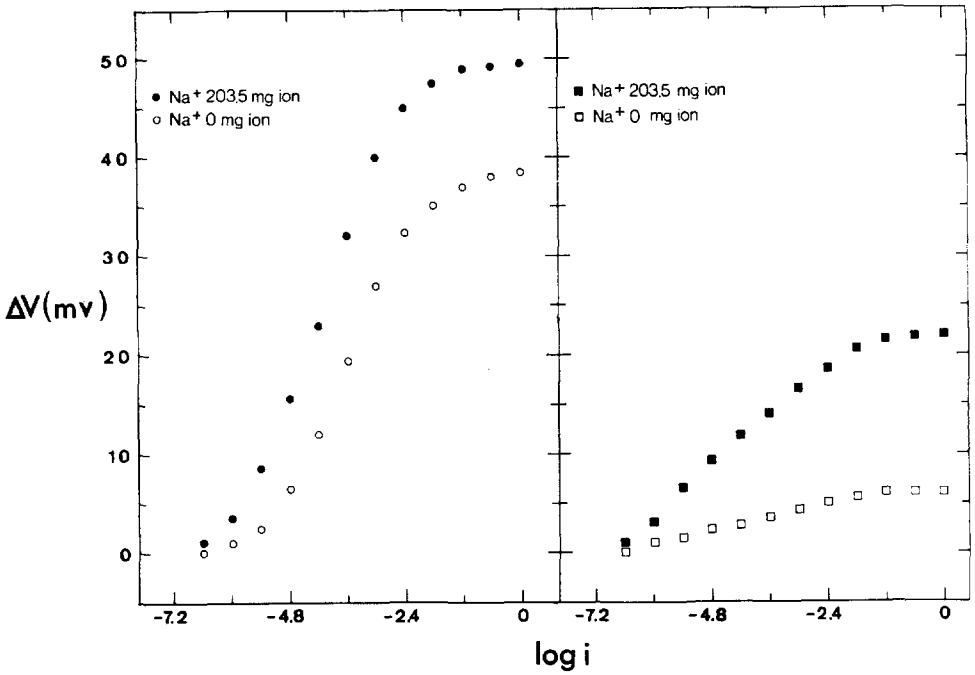

FIGURE 8. Influence of replacing Na by TrisH on the relation between voltage and light intensity. The amplitudes, (ΔV), of the transient (open and filled circles) and the plateau (open and filled squares) were measured relative to the resting potential determined immediately before stimulation. The responses obtained in the solution containing 203.5 mM NaCl (filled circles and squares) were determined before those obtained in the sodium-free solution (open circles and squares).

creased slowly and went on increasing even when the amplitude of the transient and of the plateau had reached values equal to or superior to those recorded in the normal solution. Fig. 12 a shows the shape of responses

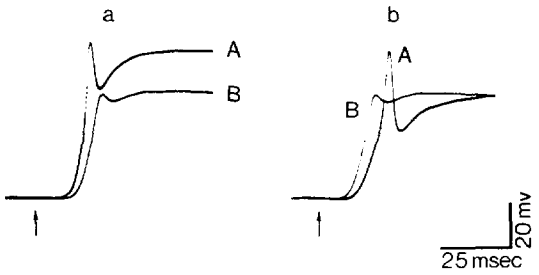

FIGURE 9. Influence of a large reduction in [Na] on the spike potential. In a, photographically superposed responses to an intense light recorded first in the physiological solution (A) and then after a short while in a solution in which Na was replaced by choline (B). In b, B is the same as in a, but this response is compared with a response to a weaker light recorded in physiological solution (A). The onset of stimulation is marked by an arrow. Stimulus duration was 1 sec.

recorded 1, 7, and 20 min after a prolonged substitution of choline for Na. It can be seen that when the plateau was high the transient was followed by a strong oscillation. When trace B is compared with trace C (identical to the response of a fresh preparation in the physiological medium), one has the

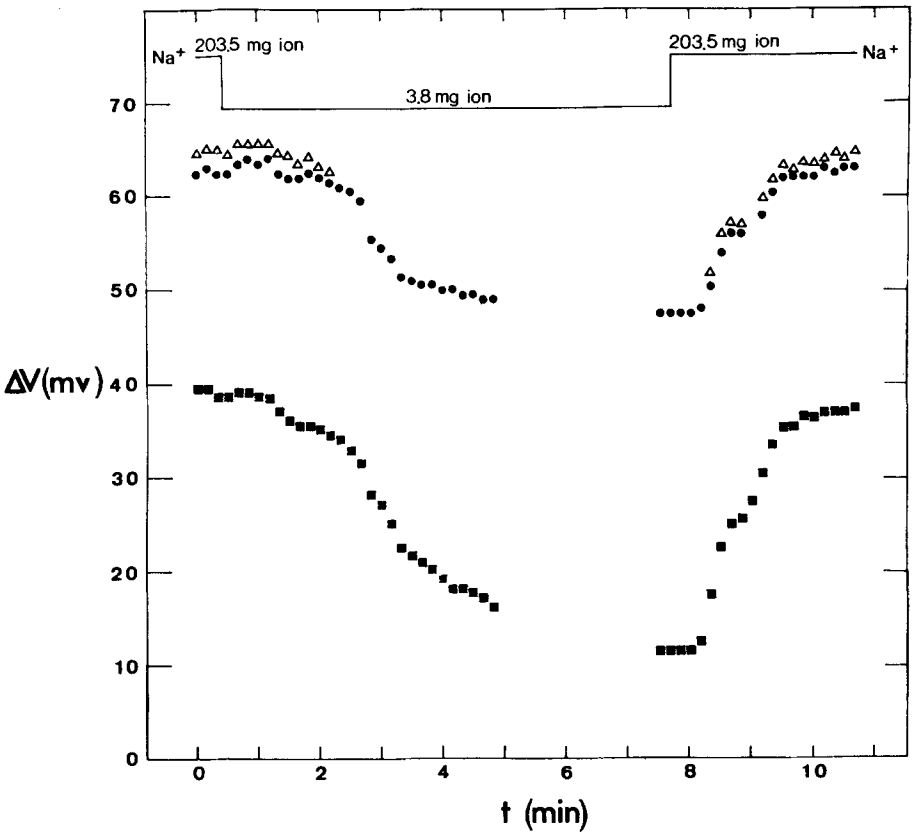

FIGURE 10. Effects of a reduction in [Na] on the amplitude of a response to intense light of 1 sec duration. Na was replaced by choline. The preparation was stimulated once every 10 sec. The amplitudes ΔV, of the transient (filled circles), plateau (filled squares), and spike (open triangles) were measured relative to the resting potential determined immediately before stimulation. Time points from the 5th to the 7th min are missing owing to the determination during this interval of the relation between voltage and light intensity.

impression that the oscillation is an accentuation of the one observed in normal responses.

Replacement of NaCl by Sucrose With this substitute (solution B, Table I) the concentration of chloride in the medium is lowered. Qualitatively the effects were, however, similar to those when choline or TrisH was used. It therefore seems unlikely that chloride plays a predominant role in the generation of the response to light. No oscillation and no overshoot of the amplitude

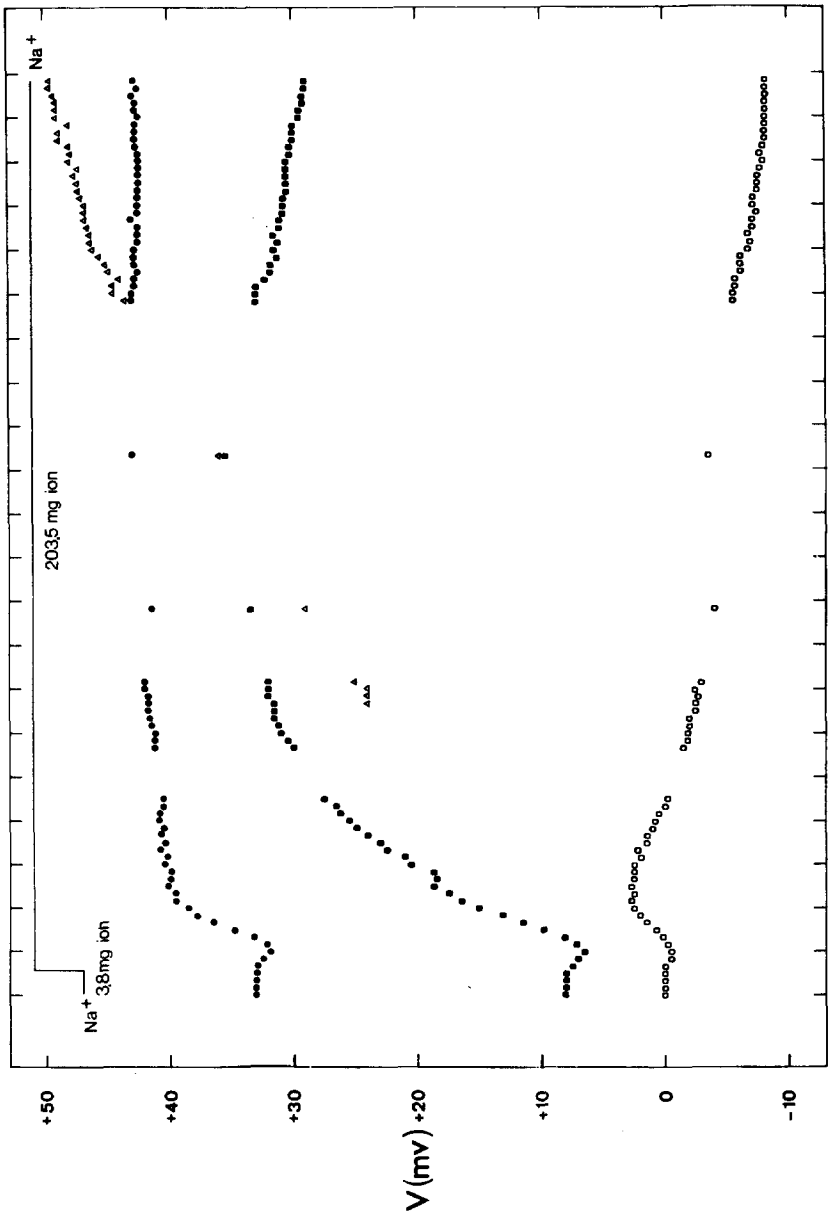

FIGURE 11. Responses to intense light of 1 sec duration recorded during the return to normal [Na] after 90 min in a sodium-deficient solution. Na was replaced by choline. The preparation was stimulated once every 10 sec. The amplitudes, V, of the transient (filled circles), the plateau (filled squares), and spike (open triangles) were measured relative to the resting potential (open squares) determined at the beginning of the experiment. The progressive hyperpolarization following the initial depolarization may have been caused by the gradual appearance of a transient hyperpolarization at the end of each response to light.

of the plateau similar to those seen in the case of TrisH or choline substitution appeared after substitution of sucrose for NaCl. Because of changes in the junction potential between the bathing medium and the neutral electrode eventual modifications in the resting potential could not be measured when NaCl was replaced by sucrose.

Replacement of Na by Li Lithium has been shown to replace sodium effectively in numerous experimental situations (Schou, 1957). This does not seem to be the case for the response of the retinula cell to light, as the form of the response was changed after total substitution of Li for Na. The changes

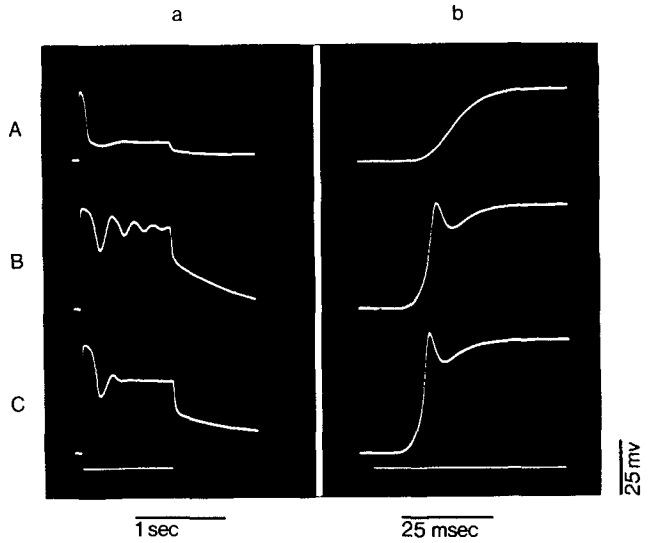

FIGURE 12. Modifications of responses to strong light stimulation during the return to normal [Na] after 90 min in a sodium-deficient solution. The responses were taken from the experiment of Fig. 11, A, immediately before changing the solution, B and C, 7 and 20 min after. In b the initial part of the response is shown at a sweep speed 40 times higher than in a.

were similar to those described for the other substitutes used: the rate of depolarization was reduced, the spike abolished, and the amplitudes of both components of the slow potential diminished, the plateau more than the transient. The effects of a replacement of Na by Li are shown in Fig. 13. Unlike the other sodium substitutes, lithium depolarized the retinula cell. This depolarization was rapid and closely resembled the one observed when [K] was increased. As a result the transient and plateau rose, though their amplitudes relative to the new resting potential were smaller. On return to the physiological medium the resting potential recovered very slowly to its original value. The transient and plateau rose again and then declined gradually. The rise shown by the plateau did not appear to be completely

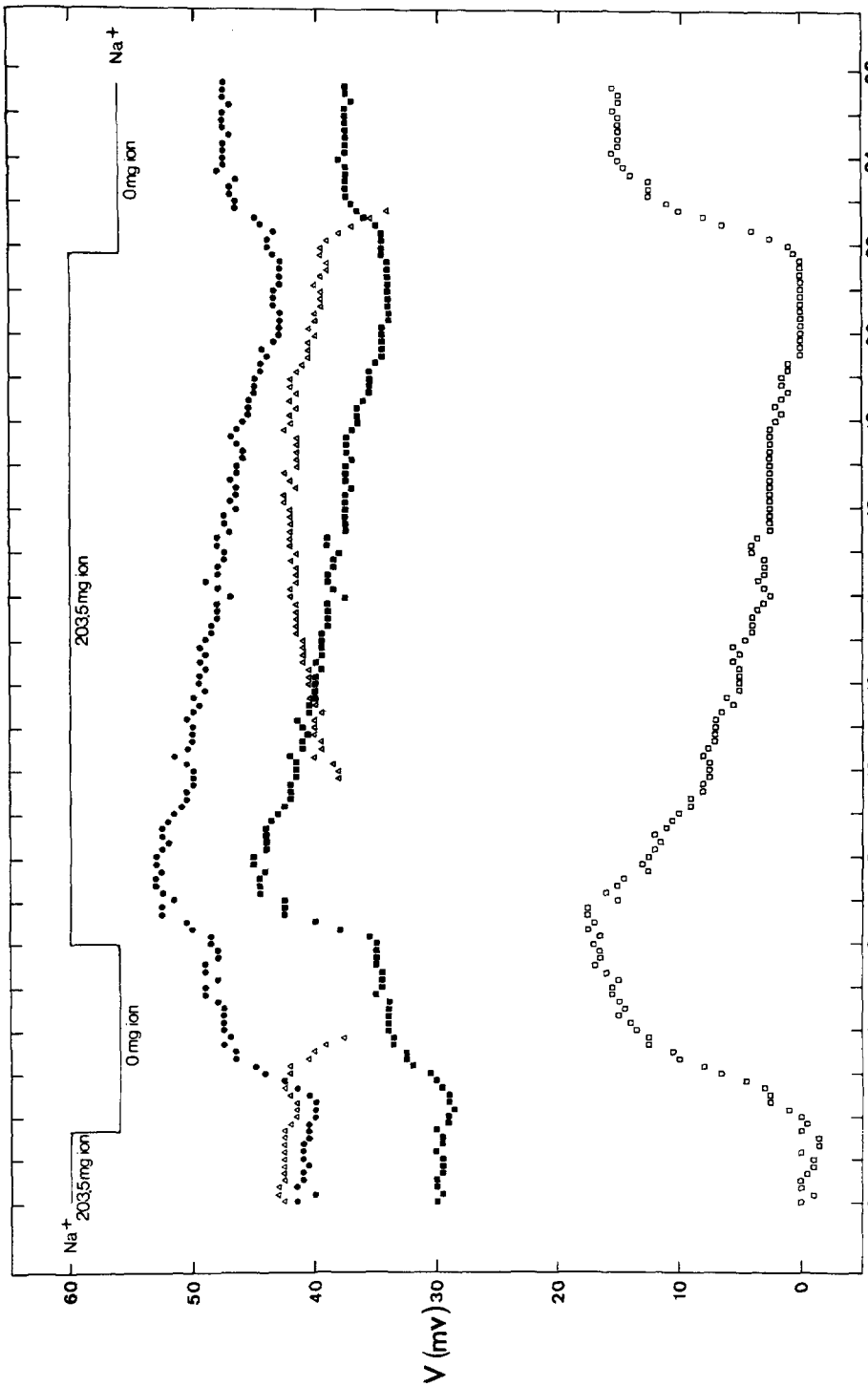

FIGURE 13. Effects of replacing Na by Li on the resting potential (filled circles), plateau (filled squares), and spike (open triangles) (open squares) of a retinula cell and on the amplitudes of its response were measured relative to the resting potential (open squares) deter- to a stimulus of medium intensity and 1 sec duration. The preparation mined at the beginning of the experiment. was stimulated once every 10 sec. The amplitudes, V, of the transient

reversible; in fact the plateau was still abnormally high in responses recorded 5 hr after return to normal [Na]. The spike reappeared in the physiological solution, but the delay between stimulus and spike maximum was prolonged. It never recovered completely.

It is clear from the results of the sodium substitution experiments that some changes in the retinula cell response to light occur whatever the substituent used. This suggests that the changes are due to the absence of sodium rather than to the specific effect of any of the substituents. The disappearance

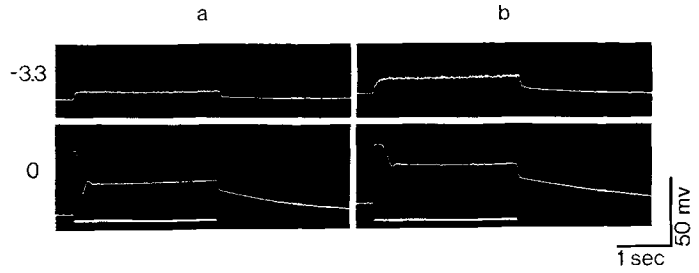

FIGURE 14 a

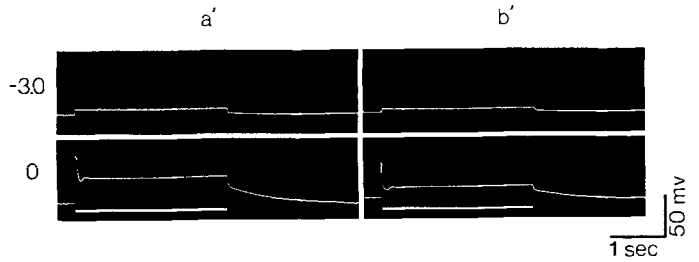

FIGURE 14 b

FIGURE 14. Effects of modifications of [Ca] on the response to weak (−3.3 and −3.0) and to strong lights (0). a and a′ are responses obtained in the physiological solution, b and b′ in solutions containing 0 mg ion/liter and 18.0 mg ion/liter Ca, respectively. Note that the responses a and a′ represent two different cells.

of the spike when sodium is absent from the medium indicates that this part of the response is due to a sodium influx. The relatively slight decline of the transient might indicate that the part played by sodium in producing this phase of the receptor potential is less important.

Effect of Calcium The membrane potential of various excitable cells is influenced by variations in extracellular [Ca]. In the squid axon this seems to be due not to changes in a calcium current but to the effect of calcium on sodium and potassium currents (Frankenhaeuser and Hodgkin, 1957). Such a mechanism could explain the modifications in the response of the drone

visual cell observed during this research. The effect of eliminating calcium from the medium is illustrated in Fig. 14 (a and b). The amplitude of the response to the low intensity light was greatly increased. The amplitude of the discrete waves, clearly visible in the record of the response in the normal medium, was greater in the absence of calcium in the medium. The amplitude of the response to a high intensity stimulus, especially of the plateau, was also greater; the transient was broader and the oscillation which followed smaller. An increase in [Ca] had the opposite effects (Fig. 14 a′ and b′). The results argue against the participation of a calcium current in the generation of the receptor potential. If such a current contributed to the depolarization induced by light, a lesser response would be expected in a low [Ca] and a greater one in a high [Ca]. If, however, depolarization following illumination

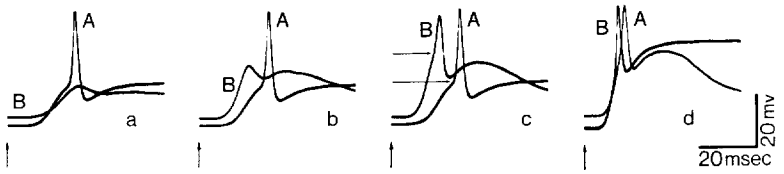

FIGURE 15. Effects of an increase in [Ca] on the spike potential. A, responses obtained in the physiological solution, B, responses obtained in a solution of 10 times higher [Ca]. Stimulus intensity in a was the same for both responses (A and B). In b, intensity of light B was increased 8 times, in c, 16 times. In d stimulus intensity was the same for A and B, but 32 times higher than in a. The onset of stimulation is indicated by an arrow at the beginning of each record. Horizontal arrows in c point to inflection points in the rising phase of the response. They indicate spike threshold. Records made in the calcium-rich solution show a slight reduction in resting potential due to the repetitive stimulation of the preparation. Traces A and B were photographically superposed in each case.

is due partly to sodium influx, the results can be accounted for by an effect of calcium on the sodium influx triggered by the light. It would be increased in the absence of calcium and diminished in a high concentration. This view is supported by the fact that the rate of depolarization is affected in the same way by either an increase in [Ca] (Fig. 15 a) or a reduction in [Na] (Fig. 12 b). Calcium appears to be without effect on the resting potential. The slight depolarization shown in Fig. 14 appeared only when the cell was stimulated repeatedly and it may be attributed to the modifications of the repolarization observed when [Ca] was changed. The effects of calcium on the spike are not easily distinguishable since in normal solution the amplitude of the spike depends on the shape of the slow potential (Baumann, 1968) and since the slow potential itself is affected by the [Ca] (Fig. 14). It was, however, possible to form some idea of these effects by working at higher light intensities, where spike amplitude is constant and no longer varies with the slow potential. The comparison of such responses in the normal and calcium-free solutions showed

that a reduction in [Ca] was usually without effect on the amplitude of the spike, although occasionally a very slight decrease was observed. A rise in [Ca] was also without effect on the amplitude of the spike, at least when the slow potential had been raised by a sufficient increase in stimulus intensity to produce a spike of full size (Fig. 15). In this figure it will be seen that in the calcium-rich solution the spike was broader and the level of depolarization needed to trigger it higher. A reduction in this level was sometimes observed in calcium-free solutions. Similar changes in threshold and shape of the spike after changes in the [Ca] have been described by Frankenhaeuser (1957) for the spike potential of a frog nerve.

DISCUSSION

The response of the retinula cell of the honeybee drone to stimulation by light consists of a slow depolarization, the receptor potential, upon which, above a certain stimulus intensity, a spike is superimposed. The results of the experiments described in this paper show that changes in the ionic composition of the solution affect the two components differently. The changes in the spike were similar to those observed in a nerve fiber, and it may therefore be supposed that the spike of the retinula cell is due to passive movement of ions through the membrane. The receptor potential is less easy to interpret. An explanation in terms of passive ionic currents is possible only if certain assumptions are made about the nature of the ions implicated in the transport of charges through the membrane and the changes in permeability caused by illumination of the cell. These assumptions have not yet been verified experimentally. The slow potential in the physiological medium, above a certain intensity of light stimulus, consists of two phases, the transient and the plateau. The amplitude of the transient rises with the logarithm of the light intensity along a sigmoid curve. Under high stimulation it reached a value close to 0 potential. This temporary abolition of the potential difference across the membrane of the retinula cell obtained with strong light can be explained if one assumes that light increases the permeability to two ions (the permeability to sodium g_{Na} and the permeability to potassium g_K for example) and that the equilibrium potentials of these two ions are equal and of opposite polarity. Such a hypothesis has been put forward by Edwards et al. (1963) for the stretch receptor cell of the crayfish; in the drone it could explain why in a potassium-rich solution (i.e. when the equilibrium potential of potassium was lowered, with the sodium potential remaining constant) the transient overshot 0 potential whereas it remained below when [Na] was lowered.

The amplitude of the plateau, like that of the transient, rises in a higher [K] (Fig. 5) and falls when [Na] is reduced (Fig. 8). In both cases, however, the variations in the amplitude of the plateau are much greater than the variations in the transient. A possible explanation of this difference is that

after the initial increase in g_{Na} and g_K, in passing from the transient to the plateau, g_K goes on increasing while g_{Na} declines rapidly. If this repolarization were caused by a change in permeability to sodium alone, or to potassium alone, the amplitude of the transient would decline more than that of the plateau when the physiological solution is replaced by a sodium-free solution. The hypothesis of a reduction in g_{Na} more pronounced than the increase in g_K between transient and plateau agrees with the observation that the resistance of the membrane is higher during the plateau phase than during the transient (unpublished observation).

A very different interpretation of the receptor potential has recently been proposed by Smith et al. (1968). These authors have found in the ventral eye of *Limulus* that removing Na or K, or increasing [Ca] in the bathing medium decreases the resting potential of the retinula cell and reduces or abolishes the receptor potential. A similar effect was obtained when ouabain at a concentration of 1 mM was added to the bath or the preparation cooled below 2°C. Since these procedures are known to inhibit active sodium transport, Smith et al. proposed that in *Limulus* the resting potential of the retinula cell is partially due to the activity of an electrogenic sodium pump. They interpreted the receptor potential as the consequence of a change in the activity of this pump. Results similar to those reported by Smith et al. have been obtained in the drone with ouabain (unpublished observation) and by lowering the temperature (Duruz and Baumann, 1968). However, as mentioned before, suppressing Na in the bathing medium or increasing the [Ca] did not change significantly the resting potential of the drone retinula cell and did not abolish the response to light. Furthermore, normal resting potentials and large responses to light were observed in preparations kept up to 9 hr in a K-free medium (unpublished observation). It would thus seem that the hypothesis proposed by Smith et al. for *Limulus* does not apply to the drone.

A difficulty in the interpretation of the receptor potential in terms of passive ionic currents alone is that the potential is not abolished in a sodium-free medium. Even after 12 hr in a solution in which all the NaCl had been replaced by sucrose, receptor potentials of an amplitude of 20 mv were observed. As sodium is the principal cation of the extracellular medium, a much more pronounced reduction might have been expected. The question which arises is why a cell subjected to prolonged perfusion with a sodium-free solution should continue to be depolarized by light. The changes in the receptor potential observed when [Ca] and [K] were altered exclude the possibility that an influx of these cations might have an important part in the transfer of charge generating the receptor potential. In the case of K, the changes in the resting potential recorded with different concentrations indicate that this ion is more concentrated within the cell. As for Ca, increasing its concentration in the medium led to a decline in the amplitude of the receptor potential.

Chloride current plays a significant part in responses to light of retinula cells of the drone. The direction of the chloride movement, however, is such that it tends to decrease the amplitude of responses to light. It has been observed (Fulpius and Baumann, 1966) that in chloride-free medium the amplitude of both the plateau and the transient is increased when light of weak or medium intensity is used; in responses to strong light, when the transient approaches 0 potential, only the amplitude of the plateau is enhanced.

A hypothesis to be considered in explaining the persistence of responses to light in a Na-free solution is that in spite of prolonged substitution, a significant amount of sodium might remain in the preparation close to the retinula cell membrane. Such a hypothesis has been put forward by several authors who have observed a persistent response in various receptor cells in Na-free solutions (Diamond et al., 1958; Ottoson, 1964; Stieve, 1964; Calma, 1965).

In the drone this hypothesis calls for special attention in view of the presence of pigment cells which almost completely surround the nonrhabdomeric part of each retinula cell. These pigment cells by actively pumping sodium into the space adjacent to the retinula cells might maintain a certain concentration of sodium at this level. It is also possible that sodium remains trapped in the electron-dense material which, as shown by Perrelet and Baumann (1969), fills the narrow extracellular space surrounding each rhabdomeric microvillus. Both mechanisms have been proposed by Treherne (1967) to explain the persistence of electrical activity in axons of the nerve cord of *Carausius* bathed in a sodium-free solution for extended periods of time. In addition it is possible that a sodium gradient is maintained across the membrane which generates the receptor potential by extrusion of sodium from the retinula cell and accumulation in its immediate proximity. The amount of sodium extruded from pigment or retinula cells might be adequate to produce a brief but relatively intense sodium influx when, after a period of darkness, the cell is illuminated and membrane permeability to sodium again enhanced. This would explain why, in a sodium-deficient medium, the early part of the response, the transient, showed a relatively slight decline and the plateau a much greater decline. The existence of such a mechanism could also explain the observation that whereas in a sodium-free medium the amplitude and the rate of depolarization of responses triggered by intense light stimuli are increased by prolonging the interval between two stimulations, this is not the case in the normal medium (unpublished observation).

It is interesting to note that effects on the amplitudes of the transient and plateau similar to those described above have also been observed in the photoreceptor cells lying along the olfactory nerve in *Limulus* bathed by a Na-free solution (R. Millecchia and A. Mauro, personal communication). In this preparation, however, before the steady state is reached, there is a transient abolition of all electrical activity lasting for a period of several minutes. This

indicates that more complicated mechanisms than those described above might be responsible for the persistence in a sodium-free medium of responses to light in photoreceptor cells. It is possible that a transient silence has not been observed in the drone due to the fact that washout of sodium was slower in this preparation (consisting of half a head) than in the small tissue fragment used by Millecchia and Mauro. Moreover, some doubt is cast on the persistence of sodium in the tissue by the finding that the spike recorded in responses to light and also to depolarizing current (unpublished) disappeared rapidly and completely after removal of sodium from the medium. In a nerve fiber it is recognized that 5 % of the normal sodium content of the bathing medium is sufficient to cause a spike to appear (Huxley and Stämpfli, 1951). If this finding applies to the drone (experiments described above with various ions have shown that the spike of the retinula cell has some resemblance to that of a nerve fiber), it would indicate that sodium has for the most part been eliminated from the preparation. To reconcile these two contradictory observations one has either to admit the existence of two membrane sites (one in which the slow potential is generated and which is not easily accessible, the other the site of the spike, readily accessible) or to postulate that some unknown mechanism other than passive ionic movement through the membrane is responsible for the generation of the receptor potential.

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