J. Physiol. (1979), **294**, *pp.* 613–626
With 6 text-figures
Printed in Great Britain

TIME- AND VOLTAGE-DEPENDENT IONIC COMPONENTS OF THE ROD RESPONSE

BY F. S. WERBLIN

From the Departments of Electrical Engineering and Computer Sciences, and the Electronics Research Laboratory, University of California, Berkeley, California 94720, U.S.A.

(*Received 21 July 1978*)

SUMMARY

1. The electrical properties of individual rods, physically isolated from the rod network, were measured in terms of the time course of response and voltage–current relations derived from current steps. Properties were measured in normal and altered bathing media designed to reveal the ionic basis for the time and voltage dependent properties of the rod response.

2. In normal media the rod membrane was strongly outward-rectifying with slope resistance near 100 MΩ when hyperpolarized, but near 10 MΩ when depolarized from a typical ambient level near 35 mV. The membrane become inward rectifying for hyperpolarizations beyond − 95 mV, with slope resistance near 70 MΩ.

3. The normal hyperpolarizing overshoot associated with the rod response was strongly potential dependent: the overshoot in response to a current step disappeared when the membrane was first depolarized or hyperpolarized by more than about 10 mV from the − 35 mV ambient potential level. The decay from overshoot, elicited either by current or light, could be approximated with a first order time constant of about 150 msec.

4. In the absence of sodium the peak–plateau sequence remained intact. Membrane resistance increased during transition to the plateau. The plateau became *more* hyperpolarized than the early phase during responses beyond − 75 mV. These results indicate a time- and voltage-dependent conductance other than sodium contributes to the peak–plateau response, probably potassium.

5. Outward rectification was greatly reduced in the presence of 15 mM-TEA, suggesting that it is mediated by potassium activation.

6. Inward rectification, and the associated transients near − 95 mV were eliminated in the presence of 2 mM-caesium, suggesting that potassium conductance contributes to the time and voltage dependent inward rectification.

INTRODUCTION

Recent studies have revealed two important properties of the rod system in some lower vertebrates. The rods are coupled, probably electrically, so the response in each rod is influenced by the activity of its neighbours (Copenhagen & Owen, 1976;

Research sponsored by the National Institutes of Health grant EY00561.

0022-3751/79/4980-0620 $01.50

Fain, Gold & Dowling, 1976; Schwartz, 1976; Werblin, 1978). In addition, the rod response consists of an initial hyperpolarizing overshoot, which decays to a sustained potential level in the presence of maintained illumination (Fain, 1976; Brown & Pinto, 1974; Werblin, 1975, 1978). The transient response and the coupling between rods lead to a surprising functional property of the system: initially the rod response to spatially limited stimuli is spread over a relatively large area of the rod mosaic, and later the area of activity contracts to a narrower region (Detwiler, Hodgkin & McNaughton, 1978).

This paper is concerned with the electrical properties and possible ionic mechanisms involved in the transient response of the rods. Coupling between rods tends to obscure measurement of the electrical properties, so individual rods have been isolated from the retina to facilitate these measurements.

The results suggest that a slow potassium inactivation may in part mediate the transient decay of response following the initial hyperpolarizing overshoot. In addition, there appears to be a separate, potassium-mediated inward and outward rectification of the rod membrane. The electrical properties of the light response will be presented in a subsequent study (F. Werblin, in preparation).

METHODS

Individual rods were separated under visual control from living retinal sections (Werblin, 1978) and then penetrated with two separate electrodes. The methods for preparing the living sections, and for penetrating the cells have been reported previously, and illustrated in Werblin (1978, Pl. 1 and 2).

Isolating rods

Rods on the surface of the sections (Werblin, 1978) often floated free from the preparation, and some, attached tenuously, could be teased away from the sections with the recording electrodes. A typical section with about 100 rods along its surface, would yield about five isolated rods. The isolated rods usually lacked the axon and synaptic terminals. They had resting potentials near -35 mV, and generated light responses, as long as they remained in contact with some part of the section. Another method of isolating rods is given by Bader, (1978).

Bathing medium

The standard bathing medium contained 108 mM-NaCl, 2·5 mM-Kcl, 2·0 mM $CaCl_2$, 1·0 mM-$MgCl_2$, and 4 mM-HEPES, adjusted to pH 7·6. In altered media, Na was fully replaced by choline, TEA was substituted for equimolar sodium, increased potassium was substituted for sodium, caesium at 2 mM was simply added to the medium, and sodium chloride was replaced with sodium methylsulphate.

Experimental limitations

The main advantage of using the isolated rod preparation was that electrical measurements were then possible since coupling was eliminated. The disadvantage was that rods penetrated with two electrodes remained viable for only about 10 min. This was sufficient time to make electrical measurements, but not for changing ionic environment away from and back to the standard. Therefore rods were studied in a particular altered media during each experimental session, and characteristic profiles in each medium were then compared.

RESULTS

The strategy of these experiments was to determine the time course of response and the current-voltage profile for the isolated rod membrane in normal ionic medium, then to compare the response time course and profile with those obtained in altered

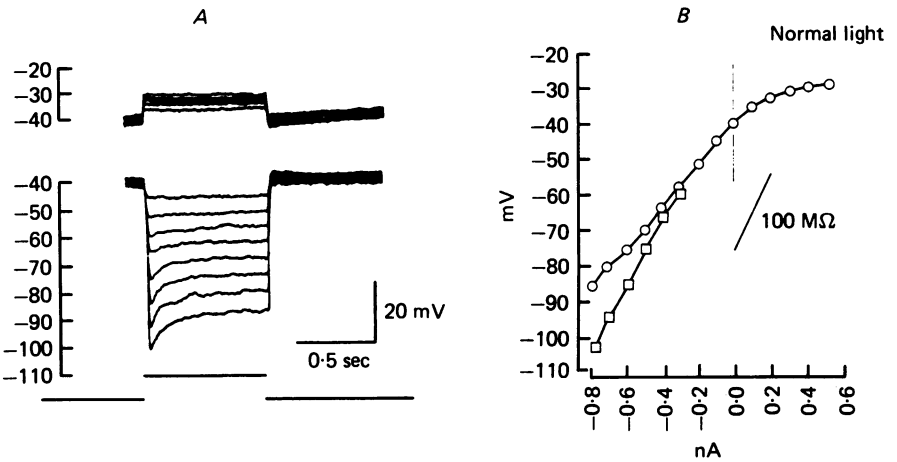

Fig. 1. Rod response to current steps in normal medium. In this and subsequent figures, part *A* shows response to current steps, injected through a separate electrode in 0·1 nA increments, both hyperpolarizing and depolarizing. Part *B* shows the voltage–current profile for early (squares) and late (circles) phases of the response. Ordinates are absolute membrane potential levels. *A*, the hyperpolarizing responses show an initial overshoot, followed by a less hyperpolarized plateau for all current steps beyond 0·2 nA. Depolarizing responses are small and 'square'. *B*, voltage current profile for rod shows strong outward rectification, and also inward rectification for response potentials beyond about 95 mV. No change in membrane resistance could be measured under normal conditions within 40 mV of the ambient membrane potential near −30 mV here, during the peak-to-plateau transition.

media. Differences in these profiles were then interpreted in terms of the ionic components presumed to be affected by the altered media. These measurements can only be performed in isolated rods, because the electrical measurements are obscured by electrical coupling between rods. However, these results are limited by the experimental constraint that individual rods could only be studied for about 10 min before they deteriorated.

All experiments reported here were carried out in the light. The brightest light that the rods were exposed to was that of the microscope illuminator. When a red filter was used, the rods were capable of generating a light response after the illuminator was turned off.

Electrical measurements in normal media

The voltage–current profile in normal ionic medium

Fig. 1 shows the response waveforms and the voltage–current profile for a typical rod in response to hyperpolarizing and depolarizing steps of current, about 1 sec long, increasing in increments of 0·1 nA for each subsequent response. The membrane potential here was about −40 mV, about 5 mV more negative than for rods in the

dark. The response waveforms were relatively square for both depolarizing and hyperpolarizing current steps for potentials within 20 mV of the ambient level. A peak-plateau sequence developed for hyperpolarizations beyond about 20 mV here.

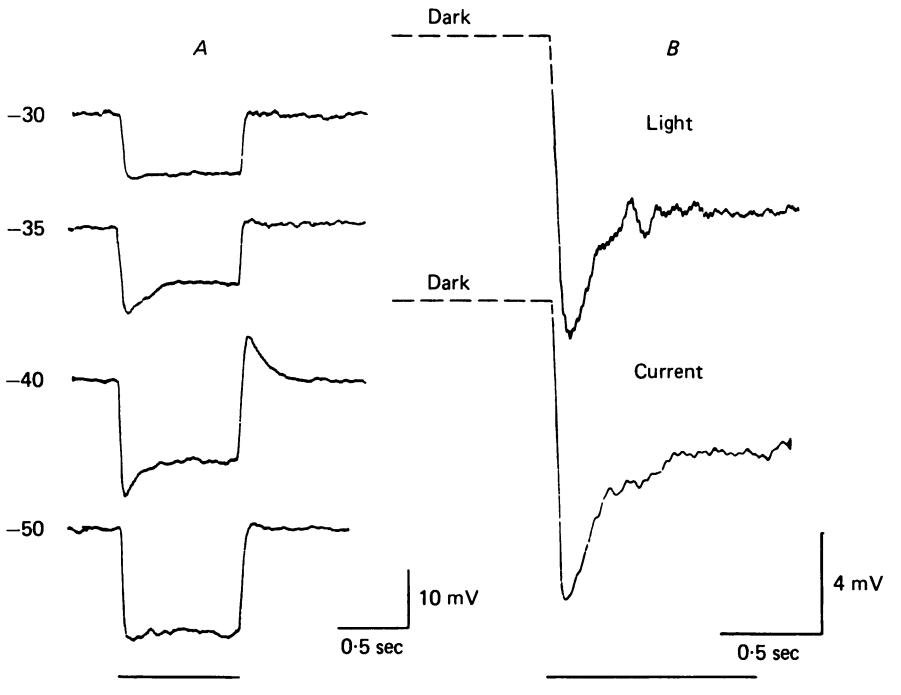

Fig. 2. *A*, peak–plateau phase depends upon ambient potential level. Responses are to 0·1 nA current steps initiated at different ambient potential levels set by steady extrinsic current, and indicated by numbers to the left of the traces. Peak–plateau phase exists at the normal ambient level, here at −35 mV, but disappears when the membrane potential is displaced to −30 or −50 mV. A peak–plateau phase can be measured at both onset and termination of the current pulse when the membrane is held at −40 mV. *B*, similarity in potential decay following response to step of light and current. In each case the initial 10 mV hyperpolarizing peak response decays to a less hyper-polarized level with an approximate first order time constant of about 150 msec. Dashed lines indicate ambient potential levels. Test flash adjusted to give near maximal response in upper trace, 0·1 nA current step used in lower trace.

The voltage–current profile for the rod is shown in Fig. 1*B*. The slope resistance for the steady-state response is near 100 MΩ for moderate hyperpolarizations, and falls to about 70 MΩ for hyperpolarizations beyond −95 mV. The membrane is also outward rectifying, with slope resistance near 10 MΩ for depolarizing responses.

The peak plateau sequence, normally measured for the light response is absent in these records because the rod is steadily illuminated, and therefore hyperpolarized beyond the normal ambient dark level. Fig. 2 will show how the appearance of the transient response depends upon the starting membrane potential.

The values for slope resistance measured here are about 3 times greater than those measured for rods in the network, and satisfy predictions about the input resistance of isolated rods, based on network properties (Werblin, 1978). However, Bader *et al.*

(1978) isolated rods with proteolytic enzymes and found input resistances near 1000 MΩ. Lasansky & Marchiafava (1974) measured input resistances near 170 MΩ in tiger salamander rods in the network, but did not find any outward rectification. Strong outward rectification has been reported in rods in mudpuppy (Werblin, 1975) and in turtle (Copenhagen & Owen, 1976).

Voltage–current profiles were determined for more than thirty rods isolated by the procedures outlined in Methods. About 50% of those rods showed response wave form and voltage–current profiles almost identical to those in Fig. 1. Many others had lower input resistances, and in those cases lacked clear cut transient components in the hyperpolarizing responses. Some rods were less hyperpolarized initially, and these often showed greater hyperpolarizing overshoots at potentials within 20 mV of the ambient potential level. These spurious results suggested that the magnitude of the overshoot might depend upon the starting potential for the response, as shown below.

Voltage-dependence of the peak–plateau sequence

The magnitude of the peak–plateau sequence in the physiological response range, within 20 mV from the ambient level, depends upon the absolute potential from which the response is initiated. Fig. 2*A* shows a series of responses to 0·1 nA hyperpolarizing current steps, initiated at different absolute potential levels. The initial levels were set by passing steady current through the membrane for about 10 sec before presenting the hyperpolarizing step.

At −35 mV, the normal potential level for this cell, the response consists of a peak–plateau sequence at the onset of the current step, but no peak at the termination of the current step. The peak component of the response was lost completely at ambient potential levels of −30 and −50 mV. Surprisingly, when the membrane potential was held at −40 mV, an additional depolarizing peak–plateau sequence appeared at the termination of the hyperpolarizing current step.

The peak component at the termination of the current step could often be augmented by slight initial hyperpolarization of the membrane. In some rods a peak response at termination of the hyperpolarizing step, or at the onset of a small depolarizing current step could be measured at the ambient potential without artificially polarizing the membrane. These results suggest that the transient response properties of the membrane may be symmetrical for hyperpolarizing and depolarizing current steps, but that the depolarizing component is usually obscured by the strong outward-rectifying properties of the membrane. More than thirty rods were studied in this way. About twenty had input resistances above 80 MΩ, and behaved as illustrated in Fig. 2*A*. The others had lower input resistances and the transient responses were smaller, or absent.

Time course of the decay of the peak

Fig. 2*B* shows typical 10 mV responses of the rod to a 0·1 nA current step and a step of light. Both show a peak, with a decay that can be approximated by a first order time constant of about 150 msec. There was some variation of these responses under different stimulus conditions. Stronger hyperpolarizations with current steps extended the time course of decay, whereas brighter light flashes often diminished the time course of the decay.

These results are consistent with the observation of Yau, Lamb & Baylor (1978) who showed that the light elicited current in individual rods in *Bufo marinus* does not appear to contain a peak, yet the normal voltage response shows a peak (Fain, 1976). Their results suggest that the peak response is generated by time- and voltage-dependent properties of the rod membrane, in response to a light-elicited current step. The isolated rods studied here never showed a peak phase in light-elicited current under voltage clamp (unpublished).

The peak-plateau sequence measured in these experiments does not involve activity at the synaptic terminal. Synaptic feed-back or voltage-dependent increases in calcium conductance (Fain, Quandt & Gerschenfeld, 1977) can be ruled out here since the rod axon and terminal have been lost in these isolated rods. Many isolated rods with input resistances above 100 MΩ lacked a measurable light response. Only five rods studied showed a large hyperpolarizing overshoot in light response as in Fig. 2*B*. All of these showed a similar overshoot in response to the current step.

Electrical measurements in altered media

Response to current steps in media without sodium

The responses to current steps, and the resulting voltage–current profile measured in a medium where sodium was replaced by choline is shown in Fig. 3. The absence of sodium has four significant effects upon the electrical properties of the rod: (1) the membrane is hyperpolarized by about 15 mV from −35 to about −50 mV (Brown & Pinto, 1974); (2) the slope resistance is increased by about 20%; and (3) the peak–plateau sequence is eliminated at membrane potentials near −70 mV. But a peak–plateau sequence reappears at voltage levels beyond −90 mV. The responses in the physiolgical range, within about 20 mV hyperpolarized from the ambient potential level still show a sign of peak followed by plateau phase. However, (4) unlike the responses in normal media, the responses without sodium are accompanied by an increase in resistance during the transition from peak to plateau. This is shown in the lower part of Fig. 3*A*.

For responses at potentials more positive than −75 mV the plateau (late phase) is more positive than the peak (early phase). However at potentials more negative than −75 mV, the late phase is more negative than the early phase. Beyond −100 mV the late phase again becomes more positive than the early phase. At potentials more negative than −100 mV, the transition from peak to plateau is once again accompanied by a *decrease* in resistance. This experiment was repeated in about ten rods, chosen for input resistance above 80 MΩ. Rods with lower input resistances usually showed smaller transients.

The presence of a peak–plateau sequence in the physiological range, even in the absence of sodium, suggests that a time-dependent modulation of some other conductance can account, at least in part, for the transient. The measurement of a resistance *increase* accompanying the positive-going transient suggests that the conductance of an ion with e.m.f. more negative than the response level is involved. The presence of a reversal in polarity of the peak–plateau sequence near −75 mV implicates an ionic system with e.m.f. near this potential level. All of these findings suggest a time dependent potassium conductance, decreasing during the transition from peak to plateau. Experiments taken in high potassium outlined below, tend to support this contention.

The recurrence of a peak–plateau sequence at responses near −100 mV and the measurement of a resistance *decrease* during the transition to plateau at these potentials, where the membrane becomes inward-rectifying, suggests another voltage- and time-dependent event. Experiments in the presence of caesium, outlined below, suggest the presence of another voltage- and time-dependent potassium conductance mediating inward rectification.

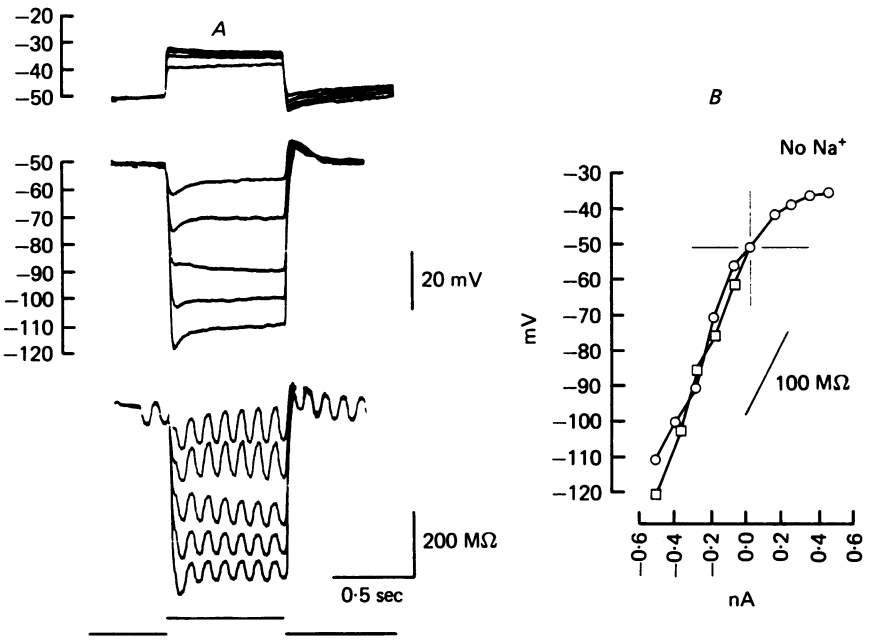

Fig. 3. Responses to current steps in the absence of sodium. *A*, membrane hyperpolarized to near −50 mV, some transient activity is measured at both the onset and termination of current steps in the physiological range. The peak–plateau sequence disappears at potentials near −80 mV, but reappears at more hyperpolarized levels. Lower traces in *A*. Membrane resistance measurements made by monitoring responses to current square wave superimposed upon the current steps, as in *A* above. The membrane resistance increases during transition from peak to plateau in the physiological range. Membrane resistance then decreases during the early–late transition for response potentials more hyperpolarized than −80 mV. These suggest an inactivation for smaller responses, but an activation for larger responses (see Text). *B*, voltage–current profile for membrane. The outward rectification and inward rectification are retained, slope resistance in the physiological range is greater than 100 MΩ.

Response to current steps in TEA

Responses to current steps measured in the presence of TEA appear to be altered in three ways: (1) the outward rectification is greatly reduced; (2) the transient component of the response to hyperpolarizing steps in the physiological range is lost; and (3) the ambient potential level is reduced from −35 mV to about −30 mV. A typical voltage–current profile, and responses to current steps is shown in Fig. 4.

Although TEA greatly reduces the outward rectification, and its presence *appears* to interrupt the transient response to current steps in the physiological range, TEA has little effect upon the transient response and rectification at membrane hyperpolarizations beyond −95 mV.

The voltage–current profile for this cell is shown in Fig. 4*B*. The outward rectification has been reduced and the slope resistance for membrane depolarization has increased from about 10 MΩ (Fig. 1) to nearly 50 MΩ. The profile for inward current resembles closely that for the rod in normal media (Fig. 1). with slope resistance near 100 MΩ, and steady-state rectification for hyperpolarization beyond −95 mV near 70 MΩ.

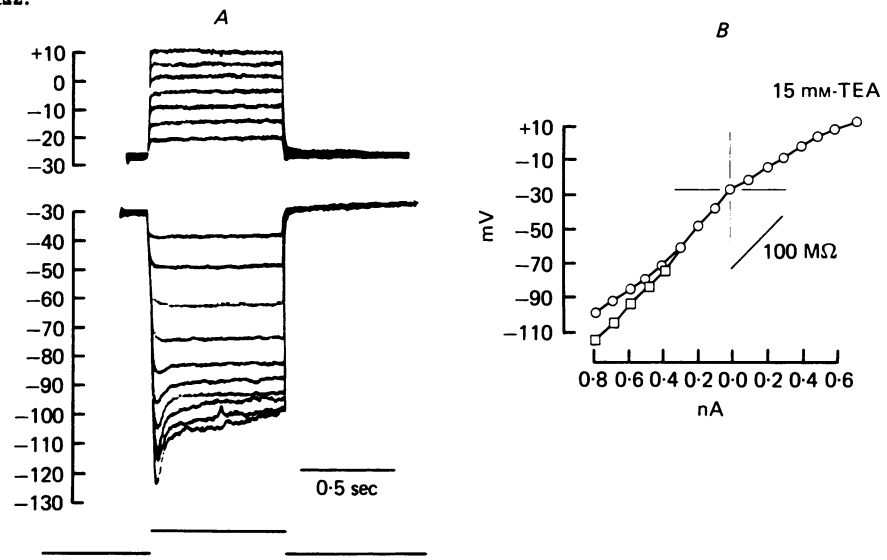

Fig. 4. Responses to current in the presence of 15 mM-TEA. *A*, membrane was depolarized to near −30 mV, the transient responses in the physiological range of hyperpolarization were lost, and the relative magnitude of the depolarizing responses to current steps increased. The transient responses at potentials beyond −95 mV again showed the transient peak–plateau wave form. *B*, the voltage–current profile lacks the strong outward rectification measured under other conditions, but retains inward rectification. Slope resistance remains near 100 MΩ.

TEA has probably eliminated the peak–plateau sequence of the response in the physiological range because the membrane is depolarized as shown in Fig. 2*A*. The transient response could usually be recovered in the presence of TEA by initially, artificially hyperpolarizing the membrane by about 10 mV to a more normal ambient potential. The change in response characteristics in TEA shown in Fig. 4 was typical of more than thirty cells studied.

From the precedents established in earlier studies of other preparations, it is reasonable to infer here that TEA is interrupting an outward rectification by interfering with a voltage-dependent potassium conductance (Katz & Miledi, 1967; Hille, 1965; Pepose & Lisman, 1978). The onset of the outward rectification appears to be too rapid to measure with the recording system in this preparation.

TEA does not appear to directly affect the slower (150 msec) outward rectification, which is normally inactivated during the hyperpolarizing response. Therefore, the slow potassium conductance in rods appear to be different from that reported in *Limulus* ventral eye (Pepose & Lisman, 1978) and mollusc (Neher & Lux, 1972), both of which were TEA-sensitive.

Depolarizing spikes and membrane oscillations, as reported by Fain, Quandt,

Bastion & Gerschenfeld (1978) in the presence of TEA are easily measured by these methods in rods in the network, but never seen in isolated rods. Since the oscillations and the axon terminals are missing in the present experiments, it is probable that the calcium-dependent regeneration is initiated at the terminals of the rods.

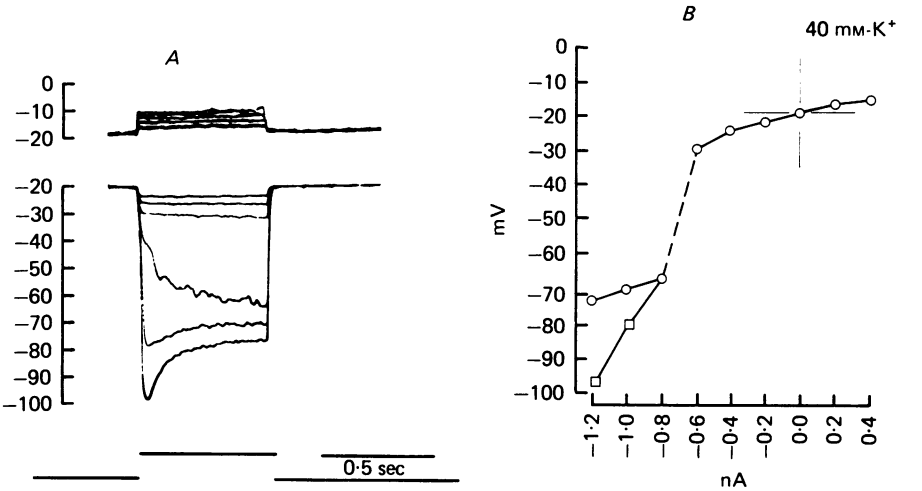

Fig. 5. Response to current steps in 40 mM-potassium. *A*, membrane is depolarized to near −20 mV, and the responses to depolarizing and hyperpolarizing currents up to 0·3 nA are small. The hyperpolarizing response to the 0·4 nA current step drifts from about −30 to −65 mV with a time constant of roughly 200 msec. For greater current steps the peak–plateau sequence apparent under all other conditions reappears. *B*, voltage–current profile shows a relatively low slope resistance, near 5 MΩ at the ambient potential level, a 'knee' for outward rectification near −30 mV, and a knee for inward rectification near −70 mV.

Response to current steps in high potassium medium

The responses to current steps, and the resulting voltage–current profile, measured in a medium containing 40 mM-potassium are shown in Fig. 5. High potassium had four significant effects upon the response of the rod membrane to current: (1) the membrane was depolarized from −35 to about −20 mV (Brown & Pinto, 1974); (2) the slope resistance in the rectification regions was reduced; (3) the knee for inward rectification was shifted from about −95 to about −65 mV; and (4) there existed a potential region from −35 to −65 mV where the membrane potential was unstable.

Responses to current steps in the presence of 40 mM-potassium are shown in Fig. 5*A*. The responses to current steps within 20 mV of the ambient potential level were 'square', showing no signs of a peak–plateau sequence. For a hyperpolarizing current step of 0·4 nA in this rod, the hyperpolarizing response slowly drifted from about −35 to near −65 mV. This experiment was repeated in many rods with similar results. The exact level at which the hyperpolarizing drift began varied from about −25 to −35 mV, and the time course of the drift also varied, but there always existed an unstable region, spanning about 40 mV, within which the membrane potential could not be controlled under these conditions.

For responses beyond −70 mV a peak–plateau sequence developed as shown in

Fig. 5*A*, and the membrane profile, for steady-state responses became inward-rectifying. It was typical for rods under these conditions to show an inward rectifying knee near −70 mV, about 25 mV more positive than in normal medium (Fig. 1). Under the same conditions the outward rectifying knee remained near −30 mV, close to the potential level for the knee in normal medium.

These results suggest that potassium is a major contributor to the ambient membrane conductance. An increase in potassium concentration from 2·5 to 40 mM should shift the equilibrium potential from a presumed value near −75 mV to somewhere near −5·6 mV, provided there is no change in potassium concentration within the membrane. However, the necessarily low external sodium concentration (68 mM) in the presence of 40 mM-potassium, could actually shift the equilibrium for sodium, another major contributor to membrane potential, to near −20 mV, assuming the sodium equilibrium potential is normally near zero mV.

The unstable region between −35 and −65 mV is consistent with a voltage-dependent potassium inactivation. In these experiments the potassium e.m.f. (−5·6 mV) is at all times more positive than the ambient membrane potential (−20 mV) so potassium inactivation will be regenerative. However, the slow drift in potential through this unstable region does not have the same 150 msec time course of the response transient under normal conditions (Fig. 2*B*). The lower limit for this unstable region, near −70 mV, could be attributed to another voltage-dependent activation, possibly for potassium, as suggested by the results below in the presence of caesium.

Response to current steps in the presence of caesium

The rod membrane appears to be affected in five ways in the presence of 2 mM-caesium: (1) the membrane is hyperpolarized to near −50 mV; (2) the peak–plateau sequence is always associated with an *increase* in resistance at all response potential levels; (3) the inward rectification is eliminated; (4) the peak–plateau sequence for responses beyond −95 mV, present under all other experimental conditions, is eliminated; and (5) there is a 20% increase in the slope resistance. Outward rectification appears to remain intact. Eleven rods, with input resistance above 90 MΩ showed the changes in wave form described below.

Responses to current steps in the presence of 2 mM-caesium are shown in Fig. 6*A*. There is some sign of a peak–plateau sequence in the physiological response range. This peak–plateau sequence reverses polarity beyond −80 mV here, where the late phase becomes more negative than the early phase. The lower recordings in Fig. 6*A* show that there is an *increase* in resistance for hyperpolarizing responses during the transition from early to late phases, even when the relative potential levels of the response phases reverse. These responses resemble hyperpolarizing inactivation measured in electroplaques (Bennett & Grundfest, 1965).

Fig. 6*B* shows the voltage-current profile for the rod. The outward rectifying knee remained intact, but the inward rectification is virtually eliminated. This rod was slightly atypical in that the outward rectifying knee fell somewhat more negative than the ambient potential level, even though the membrane had hyperpolarized to −50 mV in the presence of caesium.

Caesium has been shown in other preparations to block an inward, potassium-mediated rectification (Hagiwara, Miyazaki & Rosenthal, 1976). In those experiments the potential for onset of that rectification also appeared to be dependent upon the potassium e.m.f. Also, the outward rectification, mediated by potassium and interrupted by TEA, was not dependent upon the potassium e.m.f., a property also shared by outward rectification in the rods.

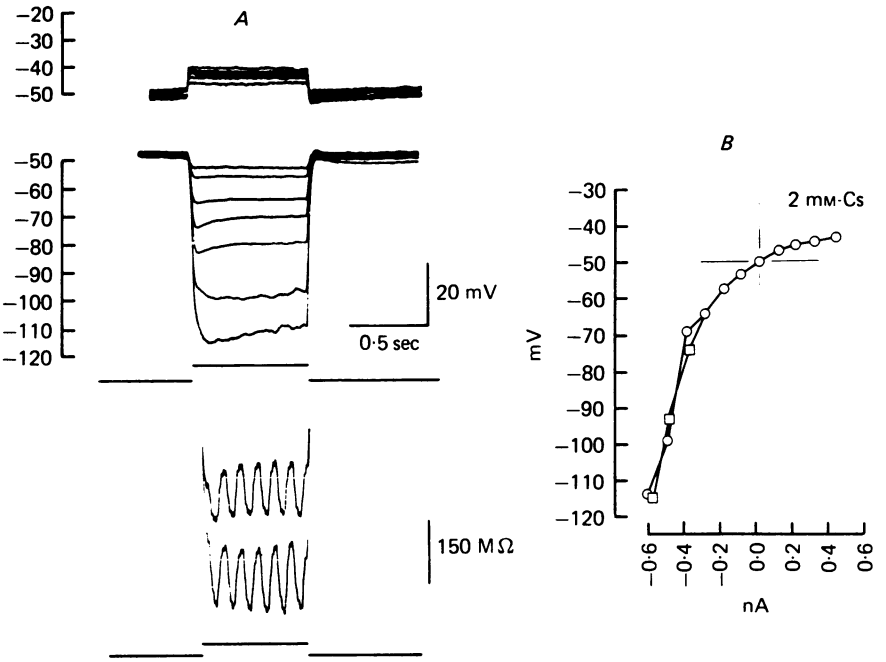

Fig. 6. Response to current steps in the presence of 2 mM-caesium. *A*, the peak–plateau response phases are apparent within the physiological range, but invert so the late phase is more hypolarized than the early phase, near − 80 mV. Lower traces show resistance measurements, like those of Fig. 4, indicating that the resistance increases here at potential levels above and below − 80 mV during the transition from early to late phase of the response. *B*, voltage–current profile shows outward rectification intact, but inward rectification is lost and membrane slope resistance increases beyond 100 MΩ for larger hyperpolarizing responses.

In addition to interfering with the potassium-mediated inward rectification, caesium also seems to interfere with the voltage- and time-dependent sodium channels. With caesium present, a resistance increase can be measured during the transition from peak to plateau. Such measurement was also possible in the absence of sodium (Fig. 3), but not under normal conditions (Fig. 1). A similar role for caesium has been suggested by Fain *et al.* (1978) in the rods of *Bufo marinus*. There, the return to plateau phase from the peak of the response was lost, and the steady-state light response was greatly augmented, suggesting a voltage-dependent sodium activation.

DISCUSSION

This paper attempts to identify and characterize some of the ionic mechanisms responsible for the voltage- and time-dependent properties of the rod response. The ionic components of the response have been inferred by comparison of the voltage–current profiles measured in altered ionic media, with profiles obtained in a normal ionic environment. In addition to the ionic mechanisms controlling the response, some inferrences have been made concerning the ionic basis for inward and outward rectification in the rod membrane, which occur at potential levels outside the range of the physiological response.

Components of the hyperpolarizing overshoot

These experiments suggest that the transition from response peak to plateau is mediated, at least in part, by a potassium inactivation with 150 msec time constant. Three results lead to this conclusion: (1) the transition to plateau remains intact in the absence of sodium, (2) the transition is associated with an *increase* in resistance, and (3) the polarity of the transition from early to late phase reverses near −75 mV.

The existence of a concommitant sodium activation cannot be ruled out by these experiments. The resistance increase and reversal of polarity of late phase measured in the absence of sodium was rarely measured in the presence of sodium. A role for calcium conductance either as a direct contributor to the transient response, or as an activator of potassium is unlikely, since the overshoot persists in the presence of 1 mM-cobalt, or in the presence of intracellularly injected EGTA (unpublished observations). A variety of slow voltage-dependent potassium conductances are known to exist in invertebrate preparations (Conner & Stevens, 1971; Neher & Lux, 1972; Thompson, 1977) as well as in spinal motoneurones (Barrett & Barrett, 1976). The inactivation phase of conductances of this sort which are not calcium-mediated in rods may account for the transient response measured in vertebrate rods.

Outward rectification

The outward rectification in rods appears to be rapid: its time course cannot be measured in these experiments. Outward rectification is blocked by TEA, as suggested previously in rods by Fain *et al.* (1977). This rectification in rods is therefore similar to outward rectification in other preparations (Katz & Miledi, 1967; Hille, 1965; Armstrong & Binstock, 1965; Pepose & Lisman, 1978), and probably represents potassium activation here as well. Although the hyperpolarizing transient appears to be eliminated by TEA in Fig. 4, it is important to note that the transient response to a current step can be restored by hyperpolarizing the membrane to −35 mV with a conditioning steady current. Therefore, TEA does not appear to directly interfere with the hyperpolarizing transient.

Inward rectification

The inward rectification, apparent for hyperpolarizations beyond −95 mV, develops with a time constant near 150 msec. This rectification normally has a knee near −95 mV, but the potential level of the knee seems to depend upon the potassium concentration in the bathing medium. Also the inward rectification is eliminated in the presence of 2 mM-caesium. These properties of inward rectification resemble

potassium mediated inward rectification described previously by Hagiwara *et al.* (1976) in the starfish egg, and by Gay & Stanfield (1977) in skeletal muscle fibres.

The responses to current steps in the presence of caesium resemble those in the absence of sodium: the transient response reverses near −80 mV and is associated with an increase in resistance. This suggests that caesium may also interfere with a component of sodium conductance in the membrane. A voltage-dependent increase in sodium conductance, (Fain *et al.* 1978), as well as the voltage-dependent potassium conductance inferred from these experiments, may contribute to the transient light response.

These results suggest that a slow potassium inactivation operating the coupled rod network, could provide the rod system with the high-pass transmission properties described by Detwiler *et al.* (1978). The potassium-mediated fast outward rectification serves to preclude spike-like regenerative depolarizations (Fain *et al.* 1978). The functional significance of the slow inward rectification is not yet clear.

REFERENCES

ARMSTRONG, C. M. & BINSTOCK, L. (1965). Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. gen. Physiol.* **48**, 859–872.

BADER, C. R., MACLEISH, P. R. & SCHWARTZ, E. A. (1978). Responses of solitary rod photoreceptors isolated from tiger salamander retina. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3507-3511.

BARRETT, E. F. & BARRETT, J. N. (1976). Separation of two voltage-sensitive potassium currents and demonstration of a tetrodoctoxin-resistant calcium current in frog motoneurons. *J. Physiol.* **255**, 737–774.

BENNETT, M. V. L. & GRUNDFEST, H. (1965). Analysis of depolarizing and hyperpolarizing inactivation responses in gymnotid electroplaques. *J. gen. Physiol.* **50**, 141–169.

BROWN, J. E. & PINTO, L. H. (1974). Ionic mechanism for the photoreceptor potential of the retina of *Bufo marinus*. *J. Physiol.* **236**, 575–591.

CONNOR, J. A. & STEVENS, C. F. (1971). Voltage clamp studies of a transient outward current in gastropod neural somata. *J. Physiol.* **213**, 21–30.

COPENHAGEN, D. R. & OWEN, W. G. (1976). Functional characteristics of lateral interactions between rods in the retina of the snapping turtle. *J. Physiol.* **259**, 251–282.

DETWILER, P. B., HODGKIN, A. L. & MCNAUGHTON, P. A. (1978). A surprising property of electrical spread in the network of rods in the turtle's retina. *Nature, Lond.* **274**, 562–565.

FAIN, G. L. (1976). Sensitivity of toad rods dependence on wavelength and background illumination. *J. Physiol.* **261**, 71–101.

FAIN, G. L., GOLD, G. H. & DOWLING, J. E. (1976). Receptor coupling in the toad retina. *Cold Spring Harbor Symp. quant. Biol.* **40**, 547–561.

FAIN, G. L., QUANDT, F. N., BASTIAN, B. L. & GERSCHENFELD, H. M. (1978). Contribution of a caesium-sensitive conductance increase to the rod photoresponse. *Nature, Lond.* **272**, 467–469.

FAIN, G. L., QUANDT, F. N. & GERSCHENFELD, H. M. (1977). Calcium-dependent regenerative responses in rods. *Nature, Lond.* **269**, 707–710.

GAY, L. A. & STANFIELD, P. R. (1977). Cs^+ causes a voltage-dependent block of inward K currents in resting skeletal muscle fibres. *Nature, Lond.* **267**, 169–170.

HAGIWARA, S., MIYAZAKI, S. & ROSENTHAL, N. P. (1976). Potassium current and the effect of caesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. gen. Physiol.* **67**, 621–638.

HILLE, B. (1965). The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. gen. Physiol.* **50**, 1287–1302.

KATZ, B. & MILEDI, R. (1967). A study of synaptic transmission in the absence of nerve impulses. *J. Physiol.* **192**, 407–436.

LASANSKY, A. & MARCHIAFAVA, P. L. (1974). Light-induced resistance changes in retinal rods and cones of the tiger salamander. *J. Physiol.* **236**, 171–191.

NEHER, E. & LUX, H. D. (1972). Differential action of TEA on two K ± current components of a mulluscan neurone. *Pflügers Arch.* **336**, 87–100.

PEPOSE, J. S. & LISMAN, J. E. (1978). Voltage-sensitive potassium channels in limulus ventral photoreceptors. *J. gen. Physiol.* **71**, 101–120.

SCHWARTZ, E. A. (1976). Electrical properties of the rod syncytium in the retina of the turtle. *J. Physiol.* **257**, 379–408.

THOMPSON, S. H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *J. Physiol.* **265**, 465–488.

WERBLIN, F. S. (1975). Regenerative hyperpolarization in rods. *J. Physiol.* **244**, 53–81.

WERBLIN, F. S. (1978). Transmission along and between rods in the tiger salamander retina. *J. Physiol.* **280**, 449–470.

YAU, K. W., LAMB, T. D. & BAYLOR, D. A. (1977). Light induced fluctuations in membrane current of single toad rod outer segments. *Nature, Lond.* **269**, 78–80.