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EFFLUX OF POTASSIUM FROM ISOLATED ROD OUTER SEGMENTS: A PHOTIC EFFECT

BY A. CAVAGGIONI, R. T. SORBI AND S. TURINI
From the Istituto Fisiologia Umana, Università di Parma, Parma, Italy

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SUMMARY

1. Illumination of the isolated outer segments of rod photoreceptors loaded with ^{42}K or ^{86}Rb reduces the efflux of these ions.

2. During the perfusion of the isolated rod outer segments with a solution containing only 2·36 mM-Na the effect of light is absent, and the amplitude of the photic effect is linearly related to the logarithm of the extracellular Na concentration.

3. In darkness, raising the concentration of K in the fluid of perfusion gives an increase of the efflux of ^{86}Rb and increasing the extracellular concentration of Ca yields a retention. The efflux of ^{86}Rb and ^{42}K is greater in darkness when sucrose or choline substitute for Na.

4. It is suggested that in darkness the isolated outer segments are permeable both to Na and to K. Light appears to decrease the permeability for Na ions. There is no evidence that the permeability for K ions is modified by light.

INTRODUCTION

In the present study the method of the ionic fluxes is applied to the isolated outer segments of the photoreceptors in order to clarify the ionic changes induced by light in these organelles. In a study of the osmotic behaviour of the outer segments, Korenbrot & Cone (1972) have shown that after isolation from the rest of the cell these organelles react to light with permeability changes. In particular they showed that the influx of Na ions was reduced by light, demonstrating that the permeability for this ion was decreased during stimulation. The use of the very different method of the fluxes of the tracer ions may strengthen these conclusions. The first aim is to detect an effect of light on the flux of tracer ions from the isolated outer segments. Since an effect has been found for the efflux of ^{42}K, and of its cheaper substitute ^{86}Rb, the second goal is to understand the mechanism responsible for this effect.

METHODS

Preparation. Freshly captured bullfrogs (*Rana catesbiana*) were kept in darkness overnight. Under dim red light the retinas were isolated and, in each experiment, a suspension of rod outer segments (ROS) was obtained by gently stroking two retinas with a very fine brush in 1 ml. Ringer solution. It consisted of entire rods, 30 % with the mitochondrial bag of the inner segment, and smaller fragments. The ROS suspension was then activated by adding an amount of radio-isotope, ^{42}K or ^{86}Rb (Camen or Sorin, and Amersham), such that the final specific activity

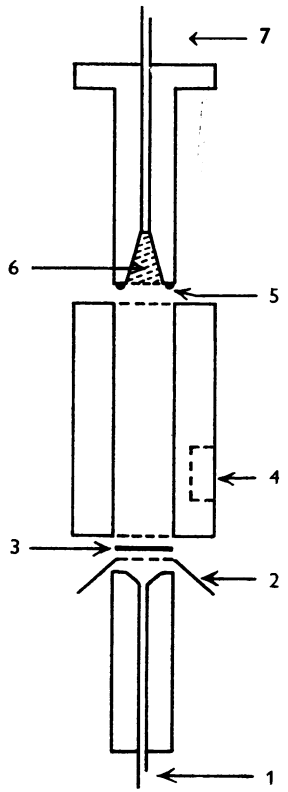

Fig. 1. Scheme of the experimental set-up. Exploded view of the perfusion chamber: 1, outlet tube; 2, nylon net; 3, millipore filter; 4, housing for the light pipe; 5, O-ring; 6, glass wool; 7, inlet tube.

was 20 μc/ml. of suspension. The ROS remained in the loading solution in darkness for 30 min. Again, under dim red light the extracellular activity was eliminated by washing and centrifuging (1500 rev/min for 30 sec) the suspension twice. The pellet was then re-suspended in 1 ml. Ringer solution, slowly injected with a syringe into a transparent perfusion chamber containing some glass-wool (Fig. 1), and successively perfused with a continuous flow of physiological solution. For the first 10 min, again in darkness, the fluid dropping out of an outflow tube was discarded. Then it was collected in samples of 5 or 10 drops, a sample of 5 drops re-

quiring about 32 sec for collection. The perfusion liquid was driven into the chamber (0·150 cm^3 volume) by a slow injector apparatus (Palmer) through an inflow tube at a rate of 0·25 ml./min.

The composition of the fluids used for perfusion is given in Table 1; the temperature was between 22 and 24° C and the pH between 7·4 and 7·6. With that chamber and the glass wool we could maintain the ROS structures throughout the entire experiment, but not perfusing the ROS directly on a millipore filter.

TABLE 1. Composition of solutions (mM)

	A	B	C	D	E	F	G	H
Choline Cl	—	—	—	—	—	—	—	—
Sucrose	—	101·80	152·70	178·16	203·60	—	—	—
NaCl	101·80	50·90	25·45	12·72	—	96·71	91·61	81·44
KCl	2·68	2·68	2·68	2·68	2·68	7·77	12·87	23·04
$CaCl_2$	1·90	1·90	1·90	1·90	1·90	1·90	1·90	1·90
$MgCl_2$	2·10	2·10	2·10	2·10	2·10	2·10	2·10	2·10
$NaHCO_3$	2·00	2·00	2·00	2·00	2·00	2·00	2·00	2·00
NaH_2PO_4	0·36	0·36	0·36	0·36	0·36	0·36	0·36	0·36
	I	**J**	**K**	**L**	**M**	**N**	**O**	**P**
Choline Cl	—	—	—	—	—	—	—	101·80
Sucrose	—	—	5·70	10·00	162·88	81·44	20·36	—
NaCl	61·08	91·60	101·80	101·80	20·36	40·72	91·62	—
KCl	43·40	2·68	2·68	2·68	2·68	2·68	2·68	2·68
$CaCl_2$	1·90	13·90	—	1·90	1·90	1·90	1·90	1·90
$MgCl_2$	2·10	2·10	2·10	2·10	2·10	2·10	2·10	2·10
$NaHCO_3$	2·00	2·00	2·00	2·00	2·00	2·00	2·00	2·00
NaH_2PO_4	0·36	0·36	0·36	0·36	0·36	0·36	0·36	0·36

Measurement of the radioactivity in the effluent fluid was made according to the method previously described (Cavaggioni, Sorbi & Turini, 1972).

The fraction of the tracer lost by the ROS per min, i.e. the rate coefficient, was computed with the following relation:

$$k_t = A_t/(\Sigma_t^{t_l} A_\tau + A_{ROS}), \tag{1}$$

where A_t and A_{ROS} are the activities of the sample collected at time t and of the ROS at the end of the perfusion respectively; t_l is the time of collection of the last sample.

The relative variation of the rate coefficient during illumination was computed and expressed in percentage. To do this we approximated the time course of the rate coefficient with an exponential: $k(t) = k(0) \exp(-bt)$, where b is the exponential coefficient of decline. In practice $k(0)$ and b were the parameters of the regression line obtained by linear interpolation of the experimental values of ln k_t which in turn were obtained when the ROS were in darkness before illumination. The relative variation was computed with the relation

$$\Delta k/k = \frac{k_t - k(t)}{k(t)} \times 100 \tag{2}$$

for the second illuminated sample. In the experiments where the efflux was changed

by test solutions, the sample yielding the maximum effect was considered and the base line was extrapolated graphically.

A retention of the tracer corresponds to negative values of $\Delta k/k$.

Other experimental details are given under Results.

Since it was difficult for us to obtain ^{42}K of sufficiently high specific activity, we used mostly ^{86}Rb which behaves like K (De Pont, Duncan & Bonting, 1971; Sorbi & Cavaggioni, 1971).

Photic stimulation was made with the same system as described elsewhere (Cavaggioni *et al.* 1972). We are unable to give an exact measure of illumination of the ROS because it was impossible to determine the absorbance of the wall of the perfusion chamber and of the glass-wool. The irradiance on the wall of the perfusion chamber is reported in logarithmic units in the text. 26·1 erg/cm^2 sec ($\lambda < 715$ mμ) corresponds to zero log units.

RESULTS

The photic effect

A suspension of isolated rod outer segments loaded with ^{42}K or ^{86}Rb slowly lost the radioactive ions in the perfusing solution of Ringer (solution A). The fraction of the isotope that was washed out by the perfusion was not constant, but slowly diminished in time. The illumination readily decreased the efflux of ^{42}K or ^{86}Rb below the normal level, and at the offset of illumination the efflux returned to the normal level (Fig. 2). The smallest irradiance on the wall of the perfusion chamber that gave a detectable effect was $1{\cdot}5 \times 10^{-2}$ erg/cm^2 sec. The isolated rod outer segments were closely packed in the filter to a thickness of a few millimetres, and the above figure is indicative only for the few rod outer segments located superficially. Those deeply hidden in the filter received much less light. The isolated outer segments were stimulated with different light intensities, to see whether greater irradiances would yield greater effects. As a general procedure the same suspension of outer segments was illuminated twice, once with a variable test light and once with a control light whose irradiance was the same in all experiments. The control was needed because of the intrinsic variability of the different preparations. The two lights were presented with an interval of 5 min and the more intense light was presented last (Fig. 3*B*). The results are reported in Table 2. The data normalized to the controls and corrected for the influence of the order of presentation are also shown.

The photic effect in low Na solutions

The suspension of isolated outer segments was perfused with a solution containing Na, 2·36 m-mole/l. (solution E) to test whether the light effect on the effluxes of K and Rb was dependent on the extracellular concentration of Na. In this condition the decremental effect of light was not present. A control illumination on the same suspension successively perfused with Ringer solution showed the effect (Fig. 3*C*). A higher concentration of

extracellular Na ions is therefore a necessary condition for the effect. Looking for the relationship between the amplitude of the photic effect and the extracellular concentration of Na, some experiments were carried out with intermediate amounts of Na in the perfusion liquid (solutions

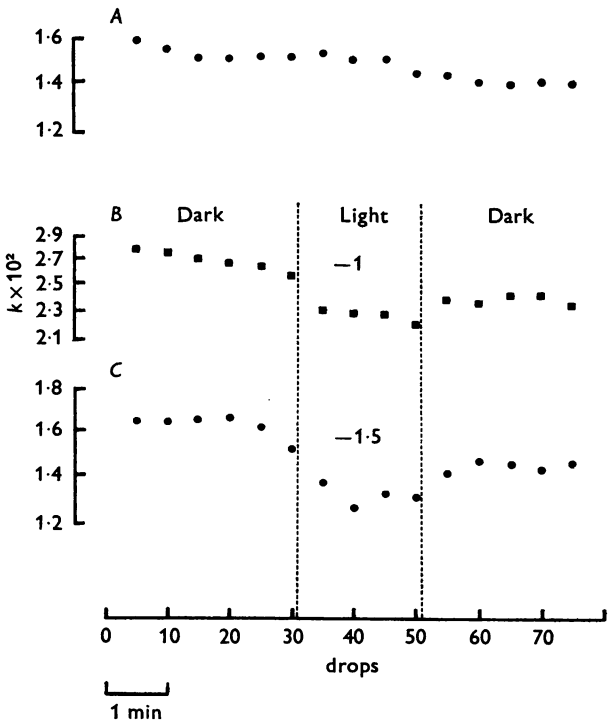

Fig. 2. Effect of light on the efflux from suspensions of rod outer segments loaded with ^{42}K (squares) and ^{86}Rb (circles). The efflux rate, k, is the ordinate on a logarithmic scale and the number of the drops collected is the abscissa. Perfusion with solution of Ringer (solution A). *A*, control in darkness, *B* and *C*, efflux during illumination. The vertical bars refer to the period of illumination taking into account the time delay of the perfusion system. Irradiance is reported in log units.

B, *C*, *D*). Here too, to account for the variability of the different preparations, each suspension was stimulated twice with an identical light intensity, the first time in the test solution and the second time in the solution of Ringer. Thus, for each suspension of outer segments a measure of the influence of the test solution on the photic effect was obtained and expressed as % of the control effect. A linear relation was obtained between the amplitude of the effect and the logarithm of the extracellular Na concentration (Fig. 4).

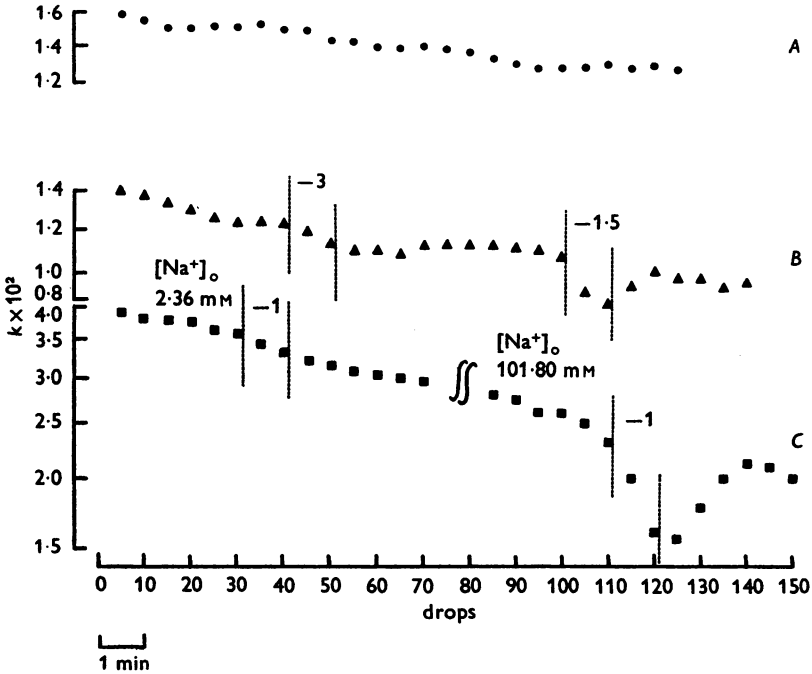

Fig. 3. Efflux rate of ^{86}Rb from suspensions of rod outer segments. *A*, control in darkness in solution of Ringer. *B*, effect of different light intensities on the same suspension. The numbers indicate the light intensity in log units. *C*, effect of low Na solution on the photic effect: the first part of this plot is obtained with Na 2·36 m-mole/l. in the perfusion fluid (solution E); observe the absence of photic effect. After 5 min the second part of the plot was obtained in solution of Ringer (solution A).

TABLE 2. Amplitude of the effect for different light intensities

Light intensities (arbitrary log units)	$\Delta k/k$ with test light		$\Delta k/k$ with control light (−1·5 log unit)		$\Delta k/k$ corrected*	
	Presented as first (Δ_s')	Presented as second (Δ_s'')	Presented as first (Δ_c')	Presented as second (Δ_c'')	$\Delta_s'\bar{\Delta}_c''/\Delta_c''$	$\frac{\Delta_s''\bar{\Delta}_c'^2}{\Delta_c'\bar{\Delta}_c''}$
0·0	—	29·7	16·5	—	—	37·9
−0·5	—	19·1	17·3	—	—	23·3
−1·0	—	20·7	16·6	—	—	26·3
−1·5	—	—	$\bar{\Delta}_c' = 16·8$	$\bar{\Delta}_c'' = 13·4$		16·8
−2·0	9·2	—	—	13·8	8·9	—
−2·5	4·9	—	—	11·0	6·0	—
−3·0	1·0	—	—	15·4	0·9	—

*$\Delta_c/\bar{\Delta}_c$ is a normalizing factor, and $\bar{\Delta}_c''/\bar{\Delta}_c'$ is a correction factor due to the fact that $\bar{\Delta}_c' > \bar{\Delta}_c''$.

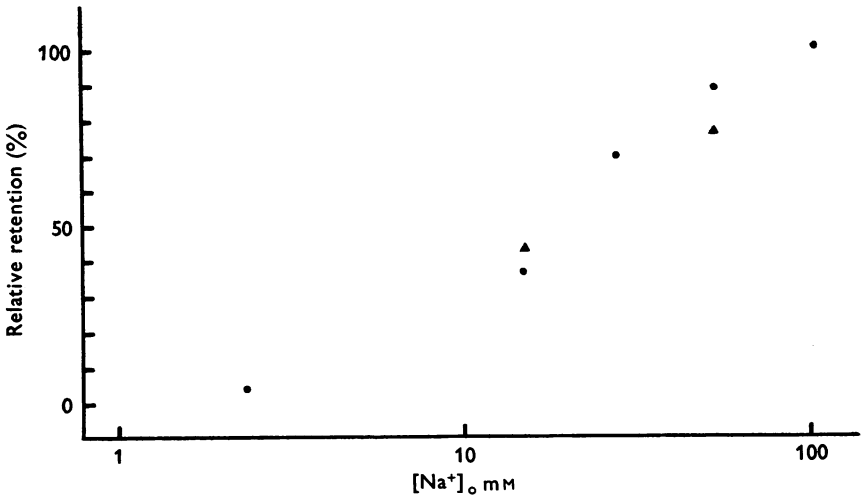

Fig. 4. Relative fraction of ^{86}Rb and ^{42}K (circles and triangles respectively) retained because of light as a function of the concentration of extracellular Na. The retention induced by light in the test solution is expressed in % retention in solution of Ringer with the same light (−1 log unit).

Effect of K, Ca and Na concentration on the efflux in darkness

The following experiment aims at showing whether a difference of electric potential may exist in the isolated outer rods segments. An attempt has been made to depolarize the isolated outer rod segments by raising the extracellular concentration of K. In fact it is known that the resting potential of the cells is reduced in this condition. The efflux of ^{86}Rb was increased by 190% when the concentration of K in the perfusion fluid was raised from 2·68 to 43·4 mM (solution I) (Fig. 5*A*). An increase of the efflux was expected to accompany the depolarization of the isolate outer segments. Smaller concentrations were also tested (solutions *F*, *G* and *H*) in order to show a relationship with the size of the increase of the efflux of ^{86}Rb (Fig. 5*B*). These results support the idea that an electric potential may be present in the isolated rod outer segments.

A decrease of the leak of ^{86}Rb was observed when the concentration of calcium ions was augmented in the perfusion liquid (solution J) whereas a physiological solution without $CaCl_2$ (solution K) induced an increased efflux of the isotope (Fig. 6). It is thus clear that the intracellular concentration of Ca is another factor that regulates the efflux of rubidium.

It was observed during the study of the effect of solutions with a low Na content on the photic effect, that the efflux in darkness was also partially modified. This point was systematically studied always in darkness

partially substituting sucrose or choline (solutions *E*, *M*, *N*, *O*, *P*) for Na. In both cases the efflux of ^{42}K or ^{86}Rb was greater. As an example, the efflux increased by 140% when the Na concentration was reduced to 2·36 mM by substitution with sucrose (Fig. 7).

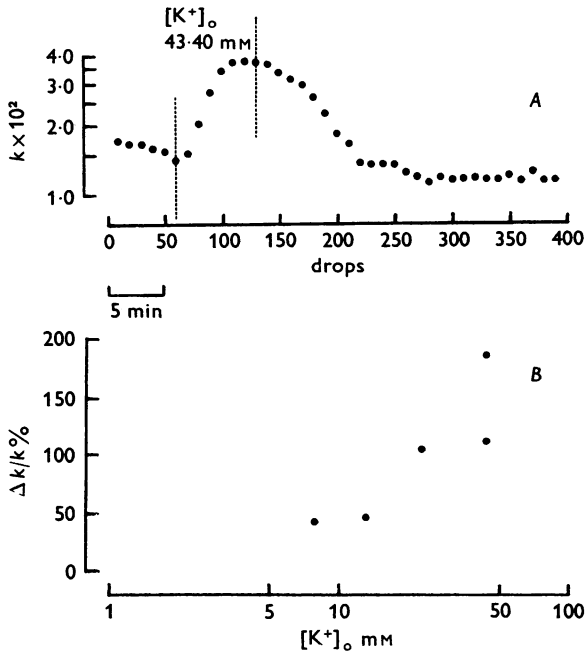

Fig. 5. Effect of high extracellular K in darkness. *A*, increase of the efflux in a solution with K 43·4 m-mole/l. (solution I). The maximum increase was determined graphically to build part *B* of this Figure. *B*, relation between the amplitude of the effect and extracellular concentration of K. The ordinate is the increase of the efflux induced by the test solution in % of the extrapolated base line. Solutions *F*, *G*, *H* and *I*.

DISCUSSION

The efflux of radioactive K and Rb from the isolated rod outer segments is modified by the action of light, i.e. the isolated photoreceptor organelles give ionic signs of excitation. For Na, a similar conclusion has been reached by Korenbrot & Cone (1972) in a study of the osmotic behaviour of the isolated outer segments of the rod cells. In both cases the intensity of light sufficient to elicit an effect is sufficiently low to make it plausible that the physiological mechanism of excitation is involved. It would be important to know which membrane gave rise to the photic effect on the efflux of radioactive K (and Rb). The material of this study consisted of rod outer segments, some of which probably sealed off after isolation, and

of shorter fragments in which the continuity of the cell membrane was presumably interrupted. In the latter case the intracellular disks that make the solid matrix of the rods were permanently in contact with the extracellular fluid. While leaving open for the time being the question as to what membrane was involved, we assume that the effects arose at the cell membrane of the isolated rod outer segments and perhaps also

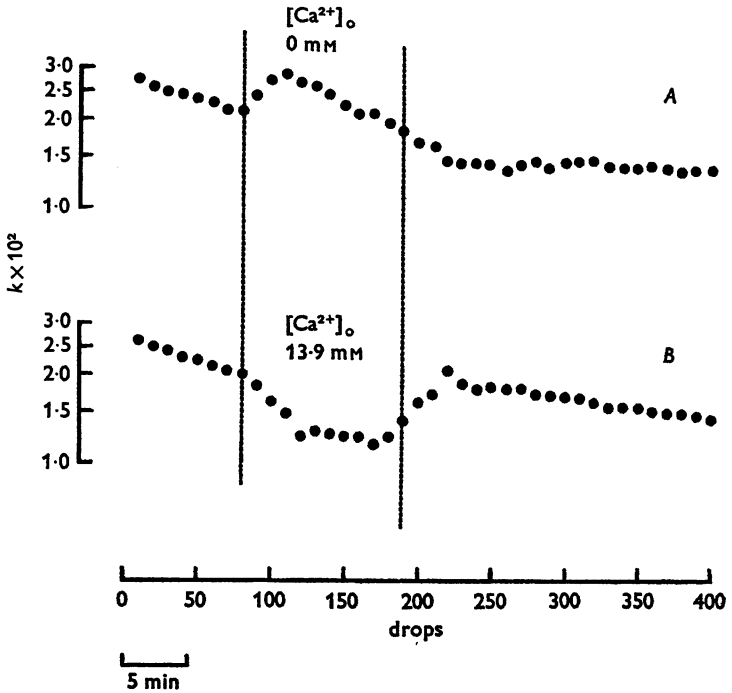

Fig. 6. Effect of varying the extracellular concentration of Ca in darkness. *A*, perfusion without Ca (solution K). *B*, perfusion with solution with high Ca concentration (13·9 m-mole/l., solution J) and Ringer solution L.

of the small bag of the inner segment that remained often attached. Indeed the modifications of the efflux of K (and Rb) brought about by the light and by the changes of composition of the extracellular fluid are consistent, at least in part, with the electrical phenomena described at the cell membrane of the photoreceptors, whilst nothing is known about the electrical behaviour of the membrane of the intracellular disks. A retention of K (and Rb) in the isolated rod outer segments occurs when they are illuminated. The effect is in agreement with the similar retention observed in the isolated retina in which the excitation of the photoreceptors has been isolated pharmacologically (Cavaggioni *et al.* 1972), but differs from

the results of Bonting & Bangham (1967) and of Duncan, Daemen & Bonting (1969) on the isolated outer segments. These results have already been criticized (Buckser & Buckser, 1971), and the present results add an additional measure of doubt. Two factors can account for the decremental effect of light on the efflux of K either a reduction of permeability for this ion, or an electric potential that retains the cations intracellularly, i.e. an hyperpolarization of the membrane. The first mechanism is less likely to

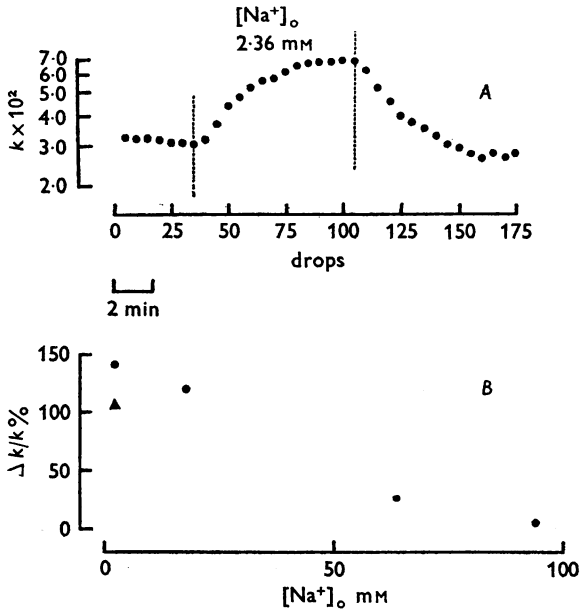

Fig. 7. Effect of low Na solutions in darkness. *A*, perfusion with Na, 2·36 m-mole/l. (solution E). *B*, relation between the amplitude of the effect and extracellular concentration of Na. The abscissa is the concentration of Na on a linear scale. Solutions *E*, *M*, *N*, *O* and *P* (triangle).

account for the absence of the photic effect when the extracellular concentration of Na is drastically reduced. The fact that the photic effect requires extracellular Na ions, and that there is a linear relationship between the size of the effect and the logarithm of the extracellular concentration of Na ions, supports the notion that light reduces in some way the membrane permeability to Na ions (Korenbrot & Cone, 1972). This in turn would produce an hyperpolarization that causes a retention of K ions intracellularly. This hypothesis implies that light modulates the electrical potential of the isolated outer segments. Intracellular recordings with micro-electrodes from these isolated organelles could add substance to this conjecture. The hypothesis that an electric potential difference

may be present across the cell membrane of the isolated outer segments is borne out by the experiments in darkness where the extracellular concentration of K was raised. In this condition an increase of the efflux of Rb was observed. This is most easily explained by assuming that the membrane was depolarized by K. On the other hand, the decremental effect on the efflux of Rb observed when the extracellular concentration of Ca was raised, may suggest that in this condition the inflow of Na is reduced and the isolated outer segments are hyperpolarized. The net inflow of Na ions in the isolated rod outer segments in darkness, however, should have been small also in a normal solution of Ringer, because they were still excitable about 50 min after isolation from the retina. In this period equilibrium of the Na activities across the membrane was unlikely to be yet attained, otherwise no excitation had been possible. In contrast, a 'dark current' probably carried by Na ions has been reported to enter the intact rods of the rat at the very fast rate of $7{\cdot}4 \times 10^{-16}$ mole/sec rod (Hagins, Penn & Yoshikami, 1970). A current of Na ions of this magnitude would equalize the intracellular and extracellular concentrations of Na in a few minutes in the isolated rod outer segments of the frog. A possibility is that an active process, about which little is known, was drawing sodium ions from the isolated rod outer segments, in order to maintain a gradient of concentration. We tested this possibility by illuminating a rod outer segment suspension which was perfused for 10 min with 10^{-5} M ouabain-Ringer solution. The photic effect was still present. Another possibility is that the permeability for Na ions was sufficiently small so as to prevent an appreciable accumulation of Na ions in the isolated outer segments during this time. Up to this point the interpretation of the data is consistent with the scheme that the cell membrane of the rod outer segments is permeable in darkness both to K and to Na ions, but that only the permeability for Na is reduced by light. One major experimental inconsistency with the proposed model should now be discussed. A *greater* efflux of K was obtained in darkness when the extracellular concentration of Na was reduced. If the membrane potential in darkness is intermediate between the K and the Na equilibrium potentials as is the case in the intact cell (Cervetto, 1973), the removal of the extracellular sodium should hyperpolarize the membrane, and cause a reduction of the efflux of cations rather than an increase. It must be concluded that the absence of Na interferes in a complex way with the efflux of K from the isolated rod outer segments.

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