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KINETICS OF ^{42}K AND ^{86}Rb LOSS FROM THE CRAYFISH RETINA IN THE DARK AND THE EFFECT OF LIGHT ON THE RATE OF ISOTOPE LOSS

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Summary

^{86}Rb and ^{42}K loss from non-illuminated crayfish retinas is described by a sum of two exponential functions with rate constants $\lambda_1 \approx 0.12$ min^{-1} and $\lambda_2 \approx 0.006$ min^{-1}. ^{86}Rb and ^{42}K movements distinguish mainly by the λ_2 rate constants, $\lambda_2^{Rb}/\lambda_2^{K} = 0.7$.

Ouabain causes a delayed increase in the rate of isotope loss from non-illuminated retinas ($\lambda \approx 0.009$ min^{-1} after 40 min). Light stimuli evoke a temporary increase in the rate of both ^{86}Rb and ^{42}K loss by the same factor (2.7 under these conditions). In the presence of ouabain the light-induced isotope loss is abolished after 40 min if the retina is periodically illuminated but not if the retina is kept in the dark.

Introduction

The membrane potential of invertebrate photoreceptors depends on ionic concentration gradients across the cell membrane (especially sodium and potassium) and on different conductances for these ion species. In the dark, the membrane potential of most invertebrate photoreceptor cells depends mainly on the external potassium concentration [1—4], and the light induced modification of the membrane potential (the receptor potential) is due primarily to an increase in the sodium conductance of the membrane [1—9], but see also [10]. To a minor extent other ion species such as calcium may contribute to the generation of the receptor potential [3,6,11]. These ideas, obtained from electrophysiological experiments predict an increase in sodium influx and in potassium efflux caused by illumination. Potassium efflux may be associated with an increase of potassium conductance but this is not a necessary prerequisite because a depolarization of the membrane potential, i.e. a change in the

electrochemical potential alone, may cause an increase in cation loss from the cell [12].

Hagins and Adams [13] studied the efflux of ^{42}K from the squid retina and reported that the efflux is slightly affected by light. De Pont et al. [14] and Holt and Brown [15] observed that illumination of both the cuttlefish retina (Sepia officinalis) and the ventral photoreceptor of Limulus leads to a considerable increment of potassium efflux. In a previous paper, Stieve et al. [16] reported that light increases the uptake of ^{22}Na and the loss of ^{86}Rb by the isolated crayfish retina. This paper continues the study of potassium movement in the crayfish retina by means of radioactive isotopes (^{86}Rb, ^{42}K).

Material and Methods

Dissection of the retina

The retina of the crayfish *Astacus leptodactylus* Eschscholz was isolated in red light following a procedure described elsewhere [17,18]. In addition to photoreceptor cells, the crayfish retina contains small foot cells and tapetum cells which account for approximately 30% of the retinal volume (refs 19 and 20 and Krebs, W., personal communication). The ommtidia are not covered by glial cells [19]. The isolated retina contains parts of disrupted crystalline cones and a small amount of nerve fibres (approx. 10% of the total volume).

Solutions

Saline. Van Harreveld's solution [21] was used as a physiological saline (Na^+, 207.3 mM/l; K^+, 5 mM/l; Ca^{2+}, 14 mM/l; Mg^{2+}, 3 mM/l; Cl^-, 244.3 mM/l; HCO_3^-, 2.3 mM/l). A modified van Harreveld's solution, used in the electrophysiological experiments, was K^+-free but contained 5 mM Rb^+/l.

Radioactive solutions. ^{86}Rb and ^{42}K were obtained as RbCl and KCl in aqueous solution (The Radiochemical Centre Ltd. Amersham). The specific activity of ^{86}Rb was between 3—6 mCi/mg Rb, that of ^{42}K was 17 μCi/mg K. Isotope was added to van Harreveld's solution to give final activities between 1—7 μCi/ml.

Procedure of wash-out experiments

The excised retina was placed on a ring (Fig. 1) which was sealed by a grid on one side. The retina remained on this ring throughout the wash-out procedure. For the uptake of isotope, the ring with the retina in place was incubated in radioactive van Harreveld's solution through which air was bubbled. The loading procedure was carried out at 13–14°C in the dark. During the manipulations a dim red light was used, otherwise the retina was kept in the dark. After the loading period the ring with the retina was washed 3 times for periods of 1 min with inactive van Harreveld's solution to remove adhering radioactive solution. The ring was then transferred to a plexiglas vessel (Fig. 1) which was perfused continously with inactive van Harreveld's solution. The flow rate of the solution was about 2 ml/min. The void volume of the vessel was 0.2 ml. The temperature of the streaming solution and of the vessel was regulated at 15°C (largest deviation ±1°C). After passage through the vessel the solution was collected with a fraction collector, the collecting period was 1 or 2 min per sample

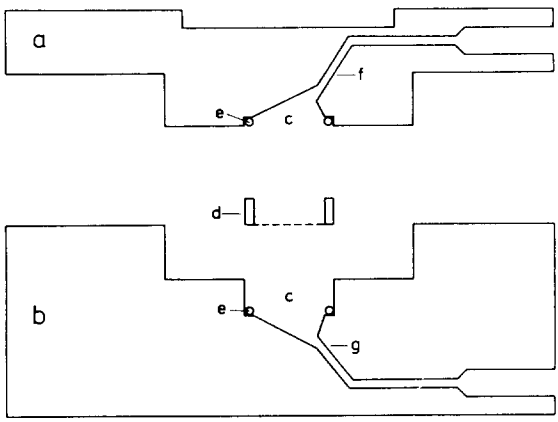


Fig. 1. Diagram of the plexiglas vessel used for the wash-out experiments. The ring d on which the retina is placed during the experiment fits into the lower part of the inner chamer c, on the lower side the ring d is closed by a grid (perlon; mesh width 80—100 μm). The light source was situated above the upper part a of the vessel. b: lower part of the vessel, c: inner chamber, volume approx. 0.2 cm^3, d: retina-supporting ring, e: O-ring, f: inlet, g: outlet.

(see Results). The wash-out procedure was carried out under a dim red light (<1 lux) except when light stimuli originating from a tungsten lamp were applied to the retina (see Results).

After the end of the wash-out procedure the retina was removed from the vessel for determination of its radioactivity. The radioactivity of all samples was determined in a liquid scintillation counter (Tri-Carb 3320, Packard Instrument Comp.). Insta-Gel (Packard) was used as a scintillator. The isotope content of the retina at a given time, $A(t)$, is then calculated from the radioactivity remaining in the retina after the end of the wash-out procedure plus the sum of radioactivity in the preceding samples.

Evaluation of double labelling experiments

The method used to determine the radioactivity when two isotopes were present in one sample is based on the different half-life of the isotope species (^{86}Rb, 18.7 days; ^{42}K, 12.4 h).

Measurement of the receptor potential

Extracellular measurement of the receptor potential was performed as described elsewhere [18].

Results

Kinetics of ^{42}K and ^{86}Rb-loss in the dark; dependence on the incubation time

Three sets of experiments were carried out to determine the kinetics of ^{42}K and ^{86}Rb-loss from non-illuminated retinas and the dependence of the kinetics on the previous incubation time. In all experiments, the retina was dark-adapted for 1 h in inactive van Harreveld's solution. It was then incubated in radioactive solution for 10 min (^{86}Rb, 5 experiments), for 3 h (^{86}Rb, 6 experiments) or

for 2 h (^{42}K and ^{86}Rb, 5 experiments). The wash-out procedure lasted for 78 min (39 collecting periods, each 2 min long). For the first 70 min, the experiment was carried out under a dim red light (<1 lux). Then the retina was illuminated for 2 min (1500 lux). Preparations which did not show an increase of isotope loss during illumination (see below) were not evaluated (less than 10% of all experiments).

Fig. 2 shows the loss of ^{42}K and ^{86}Rb from the retina in the dark. Graphical analysis of the experimental data [22] reveals that the wash-out kinetics of both isotopes may be depicted by a sum of two exponential functions: $A(t) = C_1 + C_2 = C_{10}\, e^{-\lambda_1 t} + C_{20}\, e^{-\lambda_2 t}$. $A(t)$ is the relative amount of radioactivity in the retina as a function of time. C_{10}, C_{20} and λ_1, λ_2 are the coefficients and rate constants of the exponential functions C_1 and C_2, t indicates the time in minutes from the beginning of the wash-out procedure. The coefficients and the rate constants for each set of experiments are given in Table I. The rate constants λ_2^{Rb} obtained by double labelling expeiments do not agree very well with that obtained by single labelling experiments (3-h incubation). This may be due partially to an enlarged K^+ content in the incubation medium of double labelling experiments (approx. 10 mM K^+/l). This complication, however, should be avoided by direct comparison of the data for ^{42}K and ^{86}Rb from each of the double labelled retinas. The ratio of the rate constants is: $\lambda_1^{Rb}/\lambda_1^K = 1.0 \pm 0.2$ (mean ± S.D., $n = 5$, n, no. of experiments), $\lambda_2^{Rb}/\lambda_2^K = 0.7 \pm 0.09$ (mean ± S.D., $n = 5$). There is also a slight but significant difference ($P = 0.01$) in the magnitude of the coefficients C_{10} and C_{20} for ^{42}K and ^{86}Rb (Table I). Reduction of the incubation period from 3 h to 10 min does not change the general shape of

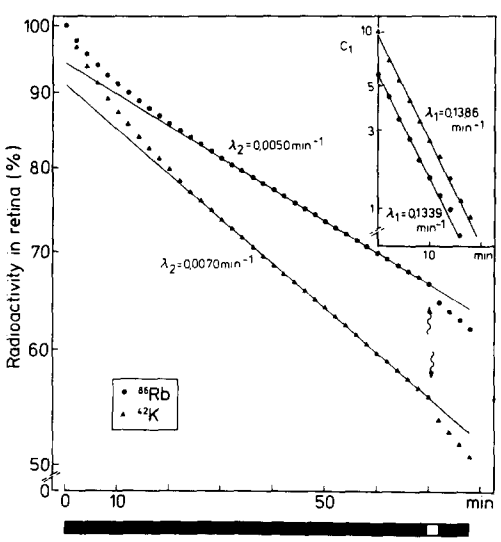


Fig. 2. Loss of ^{86}Rb (•) and ^{42}K (▲) from the isolated crayfish retina. The retina was incubated for 2 h in a solution containing ^{42}K and ^{86}Rb. The amount of isotope is plotted on a log. scale as percentage of its initial value versus wash-out time. The inset displays on a log. scale the difference between the initial phase of wash-out kinetics and the extrapolated exponential function describing the data for $t \geq 25$ min. The retina was illuminated from $t = 70$ to $t = 72$ min (↓).

TABLE I

Rate constants λ_1, λ_2 and coefficients C_{10}, C_{20} of the exponential function $A(t) = C_{10}\, e^{-\lambda_1 t} + C_{20}\, e^{-\lambda_2 t}$ describing the kinetics of ^{86}Rb and ^{42}K loss from the crayfish retina in the dark (for details see text). Mean ± S.D., n = 5—6

Experiment		C_{10} (%)	C_{20} (%)	λ_1 (min^{-1})	$\lambda_2 \cdot 10^4$ (min^{-1})
^{86}Rb	10 min incubation	6 ± 2	94 ± 2	0.10 ± 0.02	45 ± 2
	3 h incubation	5 ± 4	95 ± 4	0.12 ± 0.03	58 ± 8
^{86}Rb / ^{42}K	double labelling, 2 h incubation	5 ± 1	95 ± 1	0.11 ± 0.02	40 ± 10
		8 ± 1	92 ± 1	0.10 ± 0.03	58 ± 16

the wash out kinetics. It affects mainly the rate constant λ_2 (Table I), but it cannot be excluded that this difference may only be incidental, considering the large scattering of results and the small number of experiments.

Hagins and Adams [13] investigated the loss of ^{42}K from the squid retina. They described it as precisely exponential. Duncan et al. [23] report that the efflux of ^{86}Rb from the retina of Sepia is described by the sum of two exponentials whose rate constants are 0.46 min^{-1} and 0.02 min^{-1}. Holt and Brown [15] identified 3 exponential components in the efflux kinetics of ^{42}K from the Limulus ventral photoreceptor; a fast component (>1 min^{-1}; (estimated from (ref. 15, Fig. 5) and two slower ones with rate constants of about 0.03 min^{-1} and 0.006 min^{-1}.

The effect of light on ^{42}K and ^{86}Rb loss

From the double-labelling experiments where a test light stimulus (see above) was applied to the retina (Fig. 2), it can be seen that light stimuli increase the average rate of ^{86}Rb-loss by the same factor as that of ^{42}K, 2.7 ± 0.2 and 2.6 ± 0.2, respectively, i.e. the ratio of the average rate of isotope loss ($^{86}Rb/^{42}K$) during illumination is 0.71 ± 0.08 (mean ± S.D., n = 6).

The effect of repeated illumination on the ^{86}Rb-efflux. In four experiments the effect of light on the loss of ^{86}Rb was tested. The isolated retina was incubated for 4 h in radioactive van Harreveld's solution. During the wash-out procedure, it was illuminated for 10 s every 10 min (white light, 17000 lux).

The rate of ^{86}Rb-loss transiently increased each time that the retina was illuminated. The effect of the first light stimulus was usually stronger than that of the following ones. The increment of ^{86}Rb-loss elicited by light seems to be constant from the second stimulus onwards (Fig. 3, Table II). In one experiment, light stimuli of equal intensity but of 2- and 10-s duration were applied to the retina, the average rate of isotope loss increased to 0.74 ± 0.04% min^{-1} and 0.84 ± 0.02% min^{-1}, respectively (Table II). It should be noted that the rate of ^{86}Rb-loss is calculated for a whole collecting period which is much longer than the illumination period. Thus the true increase in the rate of ^{86}Rb-loss during illumination may be considerably larger (cf. Discussion).

De Pont et al. [14] reported that the ^{86}Rb-loss from the retina of Sepia increases up to 5-fold depending on the light intensity of the stimulus. These authors also observed that the effect of the first light stimulus on the Rb-efflux is more pronounced than that of a second one. Holt and Brown [15] investigated the effect of light on the ^{42}K efflux from Limulus ventral photoreceptor.

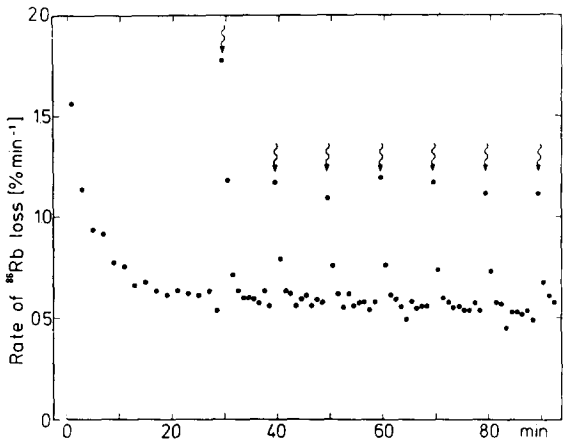


Fig. 3. Effect of light on the rate of ^{86}Rb loss from the retina. The collecting period was 2 min from the 1st to 28th min and 1 min from the 29th to 83rd min of wash-out. The retina was illuminated for 10 s every 10 min (↓) (17 000 lux, white light) beginning at t = 29 min.

The ^{42}K-loss increases up to 20-fold within 3 s after the onset of illumination and decreases again even if the light is still on.

Influence of the loading time on the light evoked increase of ^{86}Rb-loss. As mentioned above, in each experiment where the^{86}Rb-efflux in the dark was studied, a test light stimulus (1500 lux, 1 min) was applied to the retina at the end of an experiment. When the retinas were incubated in isotope solution for 10 min, the average rate of ^{86}Rb-loss was 0.43 ± 0.03% min^{-1} in the dark and 0.8 ± 0.19% min^{-1} during illumination. When the retinas were incubated in isotope solution for 3 h, the average rate of ^{86}Rb-loss was 0.58 ± 0.08% min^{-1} in the dark and 1.1 ± 0.2% min^{-1} during illumination. The light induced factorial increase in ^{86}Rb-loss was 1.8 ± 0.4 and 1.9 ± 0.3, respectively, and thus apparently independent of the length of the incubation period in the investigated range. The same result was obtained by Holt and Brown [15] on the ventral photoreceptor of Limulus.

TABLE II

EFFECT OF LIGHT ON THE AVERAGE RATE OF ^{86}Rb LOSS

Each retina was illuminated 7 times for 10 s every 10 min. The stimulus was delivered at the beginning of a collecting period (1 min) of effluent. The relative rate of ^{86}Rb loss is normalized with respect to the last collecting period before illuminarion. The data in brackets were obtained from an experiment where light stimuli of 10-s (stimulus No. 1,2,4,6) and 2-s duration (stimulus No. 3,5,7) but equal intensity were applied to the retina. Mean ± S.D., n = 4.

No. of light stimulus	Rate of ^{86}Rb loss (% min^{-1})		Relative rate of ^{86}Rb loss (per cent of dark level)	
1	1.8 ± 0.35	[1.7]	339 ± 66	[370]
2	1.1 ± 0.11	0.82	195 ± 35	160
3	1.0 ± 0.07	0.76	200 ± 33	160
4	1.0 ± 0.12	0.85	209 ± 41	190
5	1.0 ± 0.09	0.71	219 ± 38	160
6	1.0 ± 0.05	0.84	217 ± 39	200
7	1.0 ± 0.07	[0.75]	219 ± 50	[180]

Kinetics of ^{86}Rb-loss after a light stimulus. Following a light stimulus, the rate of isotope loss was transiently increased and returned to the dark level within 2—3 min (Fig. 3). A semilogarithmic plot of radioactivity in the retina and graphical analysis (Fig. 4) reveal that the loss of isotope after a short illumination may be described by a sum of two exponential functions with the rate constants λ_2^* and λ_3. λ_2^* is similar in magnitude to λ_2, the rate of isotope loss before illumination, λ_3 is in the order of 1.2 ± 0.4 min^{-1} (mean ± S.D., n = 24; n, no. of measurements).

The effect of ouabain on the rate of ^{86}Rb-loss

Effect of ouabain on the ^{86}Rb-loss from non-illuminated retinas. In five experiments, the effect of ouabain on the ^{86}Rb-loss from non-illuminated retinas was tested. Besides the application of ouabain to the retina, the experimental procedure was the same as already described; 28 min after the beginning of the wash-out procedure, the vessel was perfused with a saline containing 10^{-3} mol ouabain/l.

Ouabain evokes a time delayed increase in the rate of ^{86}Rb-loss which seems to saturate after approx. 40 min at approx. 150% of the control level (Fig. 5, cf. Fig. 6). The test light stimulus (1500 lux, 2 min) increased the average rate of ^{86}Rb-loss to about 190 ± 18% (mean ± S.D., n = 5) compared to the rate just before the light stimulus. In unpoisoned retinas, the rate of ^{86}Rb-loss increased to 180 ± 40% in response to a light stimulus of the same intensity.

Duncan et al. [23] reported that the rate constant of the slow component of ^{86}Rb-efflux from the retina of Sepia was doubled if the loading and the wash-out procedure are carried out in the presence of ouabain. However, the fast

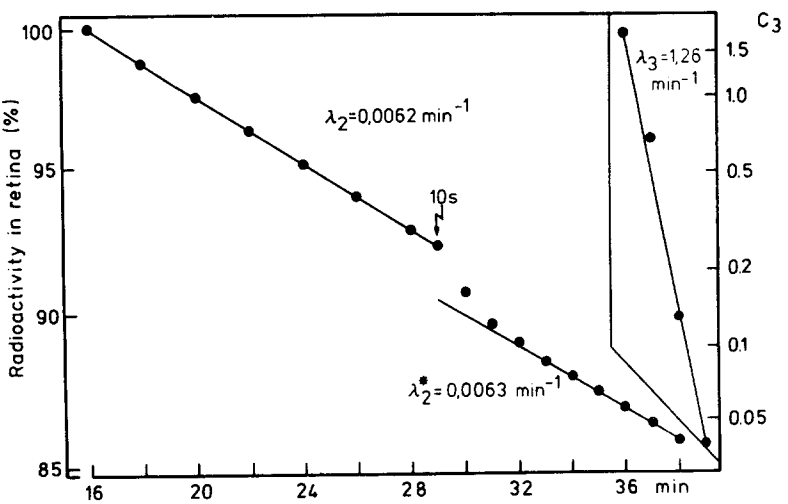


Fig. 4. Kinetics of ^{86}Rb loss after a 10-s light stimulus (first illumination of the experiment described in Fig. 3). Radioactivity in retina is plotted on a log. scale versus time. Radioactivity is normalized to t = 16 min. Time is referred to the beginning of the wash-out procedure. Before illumination the isotope loss is described by a single exponential function with a rate constant, λ_2 = 0.0062 min^{-1}. Following the light stimulus this exponential function is overlaid with another one (C_3, see inset) with a rate constant λ_3 = 1.26 min^{-1}.

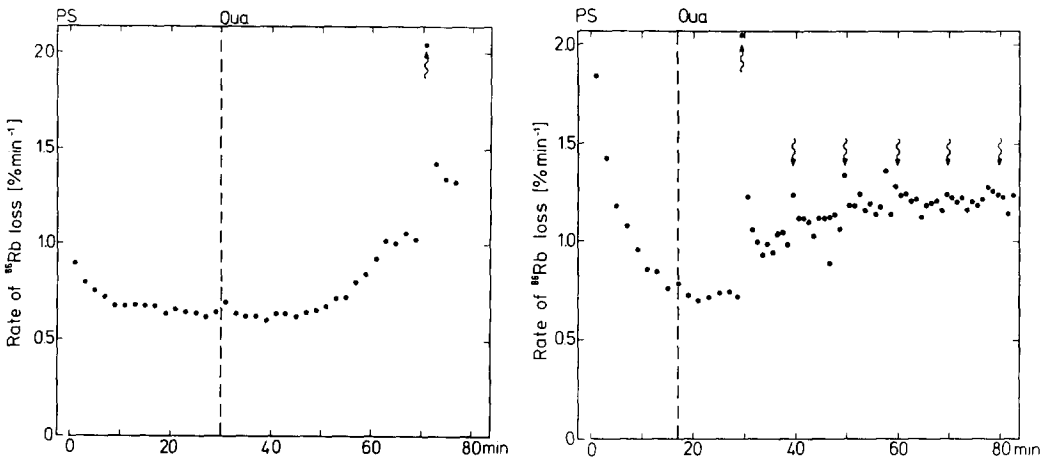


Fig. 5. Effect of ouabain (10^{-3} mol/l) on the rate of ^{86}Rb loss (cf. legend Fig. 3). During the first 28 min of the wash-out procedure van Harreveld's solution was used (PS), then a solution containing ouabain was applied to the retina (Oua). The retina was kept in the dark for the first 20 min of wash-out, then it was illuminated (↓) for 2 min (1500 lux). The collecting period of wash-out solution was 2 min.

Fig. 6. Effect of ouabain on the light induced increase of ^{86}Rb loss. For the first 18 min the retina was washed with van Harreveld's solution (PS), then a solution containing 10^{-3} mol ouabain/l was used. With exception of light stimuli (10 s, 17 000 lux), indicated by an arrow (↓), the retina was kept in the dark. The collecting period of wash-out solution was 2 min from the 1st to the 28th min and 1 min from the 29th to the 83rd min.

component was unaffected. Extended incubation in an ouabain solution (2—3 h) reduced the ability of the cuttlefish retina (even if kept in the dark) to respond to a light stimulus with a change in the rate of ^{86}Rb-loss [14].

Effect of ouabain on the light stimulated ^{86}Rb-loss. In another series of five experiments, the retina was illuminated several times in the presence of ouabain. The experimental procedure was the same as described above (cf. Fig. 3). A saline containing 10^{-3} mol ouabain/l was applied to the retina 18 min after the beginning of the wash-out procedure.

In the presence of ouabain the effect of light stimuli on the rate of ^{86}Rb-loss decreases by time and is finally no longer detectable (Fig. 6, Table III). In a similar experiment, ouabain was applied to the retina for 42 min and was then washed out again. The rate of isotope loss in the dark was 0.55% min^{-1} before the application of ouabain. 40 min later it had increased to 0.8% min^{-1}. When ouabain was washed out the rate of isotope loss decreased and reached its previous level within 50 min. Light stimuli, however, did not evoke an increase in ^{86}Rb-loss.

The difference in ^{22}Na-uptake between non-illuminated and illuminated crayfish retinas is no longer detectable after sufficient treatment with ouabain. ^{22}Na-uptake is increased in both illuminated and non-illuminated retinas [16]. Stieve et al. [24] reported that the amplitude of the receptor potential is reversibly decreased by ouabain to 30% within 27—65 min, dependent on the stimulus pattern and the light intensity. Intracellular measurements on the Limulus lateral eye have shown that, in the presence of ouabain, the receptor potential and the light induced conductance change disappear faster than the

TABLE III

EFFECT OF OUABAIN ON THE RATE OF ^{86}Rb LOSS FROM ILLUMINATED AND NON-ILLUMINATED RETINAS

t is the time after beginning of the application of ouabain (10^{-3} mol/l). Columns (a), (b) indicate the rate of isotope loss in the dark normalized to the rate just before application of ouabain. The retinas were either kept in the dark all the time (a), or illuminated every 10 min for 10 s (b), see Figs. 5 and 6. Column c describes the effect of light on the rate of ^{86}Rb loss from ouabain poisoned retinas, the rate of isotope loss is normalized to that just before illumination. Data from columns (b) and (c) are from the same experiments (cf. Fig. 6). Mean ± S.D., n = 5.

t (min)	Relative rate of ^{86}Rb loss (%)		
	a	b	c
2			260 ± 30
8	98 ± 6	98 ± 7	
12			130 ± 20
14	102 ± 9	110 ± 9	
18	103 ± 11	121 ± 15	
22			120 ± 10
24	120 ± 18	140 ± 31	
28	120 ± 20	149 ± 26	
32			110 ± 10
34	140 ± 25	150 ± 13	
38	145 ± 24	166 ± 21	
42			110 ± 10

dark potential [25]. Baumann and Mauro [26] showed that the dark potential, the receptor potential, and the light induced conductance change are abolished by light stimulation in O_2-deficient photoreceptor cells of Limulus and the honeybee drone. Moreover, they demonstrated that the recovery of the dark

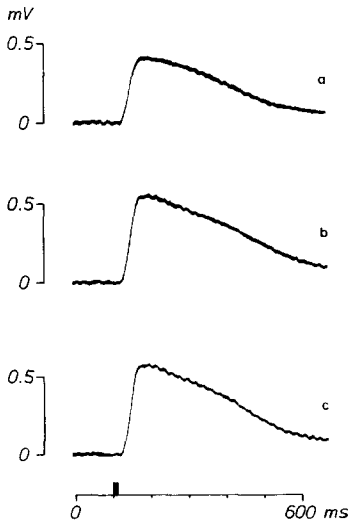


Fig. 7. Electrical responses of an isolated crayfish retina to light flashes of 10 ms duration. Receptor potentials were recorded extracellularly (see refs. 6 and 26). The retina was illuminated every 10 min. After a period of 60 min during which the retina was perfused with normal saline the preparation was exposed for 180 min to a K^+-free saline containing 5 mM Rb^+/l. The experiment ended with another 60 min perfusion with normal saline. Recordings *a*, *b*, *c* were taken at t = 50 min, t = 230 min and t = 290 min; *a* and *c* are receptor potentials before and after application of test solution.

potential has a different time course than that of the light induced conductance change.

Effect of Rb^+ on the extracellular measured receptor potential

In two experiments the effect of Rb^+ on the electroretinogram of the isolated retina was tested. A K^+-free saline containing 5 mM Rb^+/l was applied to the retina up to 3 h. As Fig. 7 shows the electroretinogram was not significantly changed by treatment with Rb^+.

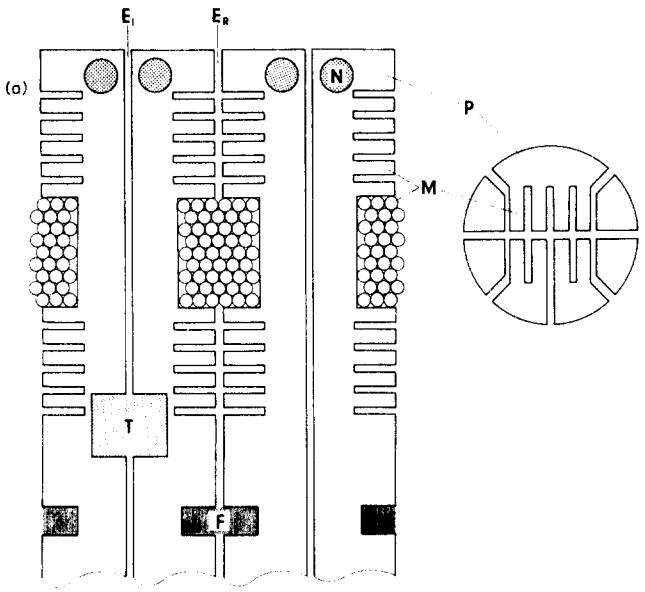
Discussion

The goal of the present and future studies is to obtain information about the distribution of K^+ in extracellular and intracellular spaces (photoreceptor cells and other cells) and to depict the K^+ exchange in these compartments if possible in terms of membrane fluxes. The double labelling experiments with ^{42}K and ^{86}Rb were performed for two reasons. The first is a more practical one. It is desirable to test if ^{86}Rb can be used as a tracer for K^+ flux since ^{86}Rb has a relatively long half-life of decay compared with ^{42}K. The second reason is that these experiments may give some information about the ion-selectivity of the photoreceptor membrane for Rb^+ and K^+. In addition, the effect of light on the exchange rate of ^{86}Rb and ^{42}K was studied because only little is known about changes of K^+ currents and possibly related changes in K^+ permeability during the process of visual excitation [1,3,9,22].

Analysis of wash-out kinetics

The wash-out kinetics describe the overall loss of isotope (^{42}K and ^{86}Rb) from the retina to the surrounding medium and in principle, contain the information about the initial isotope distribution between different compartments and about the exchange rates of these compartments. But as Fig. 8b indicates the different compartments in the retina are not independent and thus the rate constant and the coefficient of one exponential function probably contain terms from more than one compartment (see e.g. ref. 35). Another problem is that the number of possible compartments is larger than that of exponentials detected in the wash-out kinetics (Fig. 8).

It seems quite unlikely that there are exponential functions with rate constants smaller than λ_2, because the rate of isotope loss is very stable for approx. 30—76 min (approx. 30—210 min in one experiment) after the beginning of the wash-out procedure. Although no exponential functions with rate constants larger than λ_1 were detected in the wash-out kinetics from retinas in the dark, the possibility exists that such exponential functions may contribute to the observed wash-out kinetics of ^{86}Rb or ^{42}K but are obscured by the time lag of approximately 5 min between the end of the incubation period and the beginning of the wash-out procedure. One exponential function (C_3) with a rate constant larger than λ_1 was detected by the analysis of isotope loss after an illumination. However, it is possible that this component was generated or at least influenced by changes of the systems properties induced by illumination. A possible mechanism might be that illumination causes accumulation of K^+ in the extracellular space and subsequently a strong increase of the rate of active



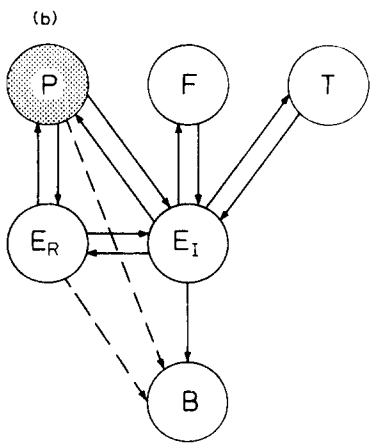


Fig. 8. (a) Schematic representation of the structure of the crayfish retina (according to Eguchi [46] and Krebs [19]). On the left a part of a longitudinal section of the retina is shown. The basic unit of the retina is the ommatidium, each consisting of 7 photoreceptor cells and the dioptric apparatus not shown here. These photoreceptor cells are arranged as indicated by the cross-section on the right. The plasma membrane of the photoreceptor cells forms extensions (microvilli) which are directed towards the center of the ommatidium. The total of microvilli of one photoreceptor cell forms the rhabdomere. The microvilli are arranged in layers. In each layer the microvilli originate in opposite photoreceptor cells, the long axis of the microvilli in one layer is perpendicular to that in adjacent layers. (b) Compartmental scheme of the retina as derived from the morphological organization. The arrows indicate pathways of ion transfer between compartments. Broken arrows indicate pathways which are expected to be of minor significance for the wash-out kinetics of isotope. B, bath solution; E_R, extracellular space in the rhabdomeric region; E_I, interommatidial extracellular space; F, foot cell; M, microvillus; N, nucleus of photoreceptor cell; T, tapetum cells.

K^+ uptake into cells. Increase of the extracellular K^+ concentration during excitation has been demonstrated for different nervous tissues [28—30] and its decline seems to depend not only on diffusion but also on metabolism [30,31].

Inspite of these unresolved problems, some general conclusions can be drawn from the present experiments. (a) A substantial amount of ^{42}K and ^{86}Rb is initially stored in cellular compartments especially in photoreceptor cells. (b) The exchange rate of cellular compartments (photoreceptor cells) with the extracellular space is probably not much larger than $2 \times \lambda_2$ (approx. 0.012 min^{-1}).

Evidence supporting conclusion (a) comes from the effect of membrane specific agents like ouabain and light stimuli on the rate of isotope loss and from the difference of ^{86}Rb and ^{42}K wash-out kinetics. It is known (see below) that cell membranes discriminate between K^+ and Rb^+, although the diffusion coefficients for Rb^+ and K^+ are nearly equal in free solution [32]. Therefore, it seems plausible that if there is an effect of light on the rate of ^{86}Rb or ^{42}K loss, some isotope should be stored in photoreceptor cells. Evidence for conclusion (b) also comes from the ouabain experiments. Fig. 8b suggests that most of the cellular compartments do not exchange directly with the bath solution but that there is an intervening extracellular space, a rough calculation indicates that probably less than 1% of the total photoreceptor cell membrane is in direct contact with the bath solution. This arrangement may result in that some of the isotope, released from cellular compartments, being taken up again by cells before reaching the bath solution. The overall rate of isotope loss from the retina may thus be reduced. In the presence of ouabain this uptake mechanism should be blocked and consequently the rate of isotope loss increased. Figs. 5 and 6 and Table III indicate that, in fact, this effect is observed and that the rate of isotope loss in the presence of ouabain approaches a value approximately twice as large as the rate before application of ouabain. Assuming that the exchange of extracellular space with the bath solution is faster than the exchange of cellular compartments with the extracellular space, we may cautiously conclude that the rate of isotope loss from cellular compartments is not much larger than $2 \times \lambda_2$ (approx. 0.012 min^{-1}).

The magnitude of the unidirectional K^+-efflux from dark adapted photoreceptor cells is calculated to be in the range of $1—5 \times 10^{-12}$ mol $\cdot$ $cm^{-2} \cdot s^{-1}$, given the following assumptions. (a) The exchange constant of photoreceptor cells is in the range, $\lambda_2—2 \times \lambda_2$ (see above). (b) The initial amount of isotope in photoreceptor cells is in the range between $0.5 \times C_{20}$ to C_{20}. This seems to be a reasonable assumption with respect to the volume of photoreceptor cells and the assumed K^+ concentration gradient between extra- and intracellular spaces. (c) The initial specific activity, cpm/mol K^+, in photoreceptor cells is between 0.5—1.0-times the specific activity of the incubation medium. This assumption is based on the kinetics of ^{86}Rb-loss and the duration of the incubation in radioactive solution (3 h). (d) The total surface area, microvillar and somatic membrane, of the photoreceptor cells is approximately 13 cm^2 (calculated from data by Bernhards [33] and Krebs [20]).

The value of K^+-efflux is used to calculate the potassium conductance (g_K) of the membrane by means of a formula given by Hodgkin [34].

$$g_K = \frac{F^2}{RT} \times \Phi \; [\Omega^{-1} \cdot cm^{-2}]$$

F, R, T: Faraday, gas constant and temperature, respectively, Φ: unidirectional K^+ efflux [mol $cm^{-2} \cdot s^{-1}$]. g_K is between $4 \cdot 10^{-6}$ and $2 \cdot 10^{-5}$ $\Omega^{-1} \cdot cm^{-2}$. This is quite similar to the electrically measured average conductance of the dark-adapted ventral photoreceptor of Limulus ($1 \cdot 10^{-5}$ to $2 \cdot 10^{-5}$ $\Omega^{-1} \cdot cm^{-2}$ [2]) and agrees well with the idea that, in the dark adapted invertebrate photoreceptor cell K^+ is the ion with the largest permeability [1—4]. There are no data on the conductance of ion channels in the crayfish photoreceptor membrane. Recent data obtained from the squid axon and frog nerve fibre indicate that the conductance of K^+ channels in the nerve fibre membrane is between $2—40 \cdot 10^{-12}$ Ω^{-1} [35—38]. If the ion channels carrying the K^+ current in the dark adapted state have a similar K^+-conductance like the K^+ channels in the membrane of the nerve fibre, than the density of these channels, open in the dark, should be in the range $10^{-3}—10^{-1}$ μm^{-2} ($10^2—10^4$ per photoreceptor cell).

Effect of ouabain on the ^{86}Rb-loss from non-illuminated and light stimulated retinas

In the presence of ouabain, the rate of ^{86}Rb-loss from retinas in the dark increases (Fig. 5). Two different explanations, not mutually exclusive, seem possible (see also ref. 14): (a) In unpoisoned retinas, the ^{86}Rb-loss is affected by the active K^+-uptake mechanism (Fig. 8b), which prevents all of the isotope released from cells to the extracellular space from being exchanged with the bath solution. However, in poisoned retinas, the rate of ^{86}Rb-loss from the retina increases because the active uptake mechanism for K^+ is blocked. (b) Blocking of the transport ($Na^+ + K^+$)-ATPases by ouabain is followed by a slow breakdown of the ionic concentration gradients which maintain the membrane potential. Depolarization of the membrane may cause an increase of K^+-efflux.

A decision between these two explanations is presently impossible. There are also some indications that ouabain in small concentration lowers the resistance of the cell membrane and increases the light response [24,25].

Although the rate of isotope loss does not increase significantly faster in illuminated retinas compared to non-illuminated ones (Table III) the ability to respond to a light stimulus by means of an increase of ^{86}Rb-loss is nearly abolished in illuminated retinas after some 30 min (Fig. 6, Table III) whereas a quite normal light response can be elicited even after 40 min application of ouabain if the retina is kept in the dark. Independently of the two mechanisms proposed for the effect of ouabain on the rate of isotope loss this result may support the experiments by Baumann and Mauro [26] and Stieve [22] on the O_2-deficient honeybee eye and the ouabain poisoned Limulus lateral eye which show that the receptor potential and the light induced conductance changes are relatively independent of the membrane potential. Whereas the loss of the receptor potential might be explained by exhaustion of the sodium concentration gradients [25], this is hardly sufficient to explain the effect on the light-induced conductance change. Therefore Baumann and Mauro [26] suggested that depletion of ATP resulting from hypoxia may be the primary factor in the impairment of the photodonductance mechanism. However, it seems difficult to apply this concept to photoreceptor cells poisoned by ouabain since ouabain is not expected to cause depletion of ATP.

Selectivity of the photoreceptor cell membrane for K^+ and Rb^+

The double labelling experiments indicate that the wash-out kinetics from dark-adapted retinas and the effect of light on the rate of isotope loss are very similar for ^{86}Rb and ^{42}K. If it is assumed that the differences in wash-out kinetics are due mainly to the passive properties of the cell membrane, whereas all other parameters determining the kinetics of isotope loss are equal for ^{86}Rb and ^{42}K, the ratio, $\lambda_2^{Rb}/\lambda_2^{K} = 0.7$, should be proportional to the ratio of K^+ and Rb^+-efflux and thus to the ratio of permeability coefficients, P_{Rb}/P_K. The diffusion coefficients of Rb^+ and K^+ in free solution differ by not more than 3% [32] and it is assumed that the relation is similar in the extracellular space. It is also not expected that the different rate constants of Rb^+ and K^+ depend on the different affinities of the active K^+-uptake mechanisms because Rb^+ and K^+ are nearly equal in affecting the ($Na^+ + K^+$)-ATPase of crab nerve and squid retina [40,41]. The ratio $\lambda_2^{Rb}/\lambda_2^{K} = 0.7$ agrees with the Rb^+/K^+ selectivity of various excitable membranes [42—45].

The effect of light on the potassium loss

Generally, the results already described confirm the findings of DePont et al. [14], Holt and Brown [17] and Stieve et al. [16] that light stimuli evoke an increase in ^{42}K or ^{86}Rb-loss from invertebrate photoreceptors. The average rate of isotope loss is considerably increased by illumination and it is probably larger than reported here (Table II) because the collecting period of effluent was considerably larger than the illumination period and because the experiment where the effect of two different illumination periods was compared suggests that the rate of isotope loss during illumination is time dependent with a maximum shortly after the beginning of illumination (Table II) as it could be shown for the ventral photoreceptor of Limulus [15].

Although it seems quite plausible that the light evoked increase in isotope loss indicates a change in potassium efflux from photoreceptor cells there are difficulties in establishing a quantitative relationship between the light stimulated changes of the isotope loss from the retina and the increase of K^+-efflux from photoreceptor cells. As indicated in Fig. 8b, it is assumed that most of the isotope loss from the retina occurs via the extracellular space. Hence changes of the rate of isotope loss from the retina reflect changes of the specific activity in the extracellular space. To determine the relationship between the rate of isotope loss from the retina and the K^+-efflux from photoreceptor cells, information about the size of the extracellular space and the time course of the specific activity of the extra- and intracellular spaces is necessary.

It can be seen from Fig. 3 and Table II that the effect of the first light stimulus on the rate of isotope loss is much stronger than that of the following ones. This may reflect a decrease in sensitivity to light of photoreceptor cells which were in the dark for 3 h before the first illumination but it could also indicate that the first light stimulus increases the active K^+-uptake mechanism thus reducing the "apparent" effect of following light stimuli on the rate of isotope loss.

Conclusive remarks

The half-time of K^+-turnover in the crayfish photoreceptor cell is probably in

the range of 60—120 min. This is a fairly high turnover compared with the squid axon and is probably due to the large surface area whose effect is only partially compensated by a low specific conductivity. The high rate of K^+-turnover may at least partially explain the sensitivity of photoreceptor cells to agents which block metabolism or active transport of ions. It would be tempting to speculate about a light induced changes of K^+-permeability because the increase of isotope loss can be impressive, but as long as the relation between changes of overall isotope loss from the retina and from photoreceptor cells is not established calculations which are based on the overall rate of isotope loss may lead to an overestimation of light induced flux changes. From the double labelling experiments with ^{86}Rb and ^{42}K which demonstrate that the ratio of the rate of isotope loss is the same in the dark and during illumination it may be concluded that if there are additional ion channels (K^+-channels) opened during illumination these channels posses probably similar discrimination properties as that opened in the dark.

Because the main difficulties which prevent a meaningful analysis of the wash-out kinetics in terms of membrane fluxes are due to the complexity of the retina and the interrelations of its constituents it will be attempted to separate the retina in subsystems which can be investigated independently. Two methods which may help are the isolation of cells and the study of the kinetics of substances which do not penetrate cellular compartments

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