SHORT COMMUNICATIONS

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Active sodium extrusion in the isolated frog retina

Although ionic fluxes in biological tissues have been studied by many investigators, relatively little has been reported on ionic fluxes in the retina. HAGINS[1] showed that in the squid rod receptors the ionic composition is similar to that in nerve cells. HAGINS AND ADAMS[2] proposed that the receptor current produced after illumination near the site of light absorption in the squid photoreceptor consists of an influx of Na^+ into the outer segment as well as an outward flux of K^+ from the rest of the cell. These results suggest that the behavior of the photoreceptor is similar to that of a nerve after excitation (*e.g.* a movement of ions across the photoreceptor membrane following visual excitation). BUCKSER AND DIAMOND[3] reported that after incubating isolated frog retinas in Ringer's solution containing ^{22}Na and then illuminating the retinas, there was an influx of ^{22}Na in the retina. BONTING AND BANGHAM[4] reported that immediately after illumination, a sucrose suspension of cattle rod outer segments lost less Na^+ and more K^+ than a control suspension that was not illuminated. Their claim that these findings are similar to what happens in a nerve after stimulation is unsubstantiated since they did not show an uptake of Na^+ after light stimulation. They merely showed that the stimulated rod outer segments lost less Na^+ than rod outer segments that were not stimulated.

The presence of a high activity of (Na^+-K^+)-activated ATPase has been reported in the rod outer segments[5]. This activity suggests that after illumination the ionic gradients in the photoreceptor cell could be restored by active Na^+ extrusion and K^+ uptake. This report provides experimental results demonstrating an active Na^+ extrusion process in the retina.

Retinas were excised in dim red light from frogs that had been dark adapted overnight. The retina from one eye was used for the experiment and the retina from the other eye was used as a control. After incubating the retinas for 2 h in the dark in frog Ringer's solution containing tracer quantities of ^{22}Na, the retinas were dipped in a chamber through which unlabeled Ringer's solution flowed to remove the ^{22}Na adhering to the retinal surface; the retinas were then transferred to a counting tube containing 1 ml of fresh Ringer's solution. The methods used in handling and transferring the retinas are described in detail elsewhere[6]. After counting the gamma radiation for 2.5 min, the retinas were transferred back to the incubating solution containing the ^{22}Na for an additional 30 min, dipped in the flow chamber, and then transferred to a second counting tube containing 1 ml of unlabeled Ringer's solution. This was done so that the ^{22}Na leaving the retina during the initial counting period could be replaced, and the amounts of ^{22}Na in the retina while it was in the dark could be compared with that in the retinas after illumination. One retina was illuminated for 10 sec with white light having a radiance of 1 W/cm^2, then immediately removed to a third tube containing 1 ml of unlabeled solution. The ^{22}Na in the third

tube containing the retina, as well as the ^{22}Na remaining in the first, second and third tubes after removal of the retina, was measured. The same process was carried out for the control retina except that the control retina remained in the counting tube in the dark for 10 sec before being transferred to another counting tube. In other experiments the retinas were kept in the counting tubes 1, 2, 3, 5, and 15 min after illumination before transferral to another counting tube. The control retinas were kept in the counting tubes in the dark for corresponding periods of time before transferral to the third tube. Each experiment was repeated ten times.

TABLE I

RETINAL ^{22}Na CHANGES AT VARYING TIMES AFTER ILLUMINATION

	A (^{22}Na *remaining in retina*)* *Time after illumination (min)*					*B* (^{22}Na *efflux from retina*)* *Time after illumination (min)*				
	0	*1*	*2*	*3*	*5*	*0*	*1*	*2*	*3*	*5*
	3.4	1.7	−4.9	3.6	7.3	12.6	−0.8	9.0	−24.3	−17.4
	−2.8	−4.0	−6.1	3.5	7.1	9.0	15.0	22.6	−19.4	−18.2
	−1.7	−4.5	6.9	2.4	8.6	16.9	25.1	−11.9	− 6.5	−11.5
	−0.9	−0.2	7.3	1.9	5.9	12.5	13.1	11.7	20.9	−13.9
	−2.9	3.1	3.7	3.2	6.2	10.1	22.5	− 0.5	−18.7	−19.0
	−1.6	−0.4	−1.9	1.9	9.1	11.5	7.1	2.7	−14.9	−14.7
	−3.2	−2.1	−3.1	2.9	8.1	13.7	13.4	9.0	−17.6	−16.7
	−0.8	−1.6	5.2	2.5	6.9	9.9	15.4	−13.5	−20.3	−16.3
	−1.3	−3.1	−6.1	3.0	7.4	9.1	9.6	8.3	−20.6	−20.1
	−1.1	−2.1	4.2	3.0	2.7	12.5	18.7	11.0	−12.6	−12.8
Mean	−1.3	−1.3	0.5	2.8	6.9	11.8	13.9	4.8	−13.4	−16.1

* See text for explanation.

A direct comparison of the efflux of ^{22}Na remaining in the retinas after the first incubation is not valid, since the amount of time that the retinas remained in the first and second vials is not equal. To meaningfully interpret the data it is necessary to compare the effluxes, or retinal ^{22}Na, obtained from the illuminated retinas with those obtained from the control retinas. The results are shown in two ways in Table I. In Section A, the fraction of ^{22}Na remaining in the retina varying periods of time after illumination is subtracted from the same fraction obtained from the control retinas. At zero time, and 1 min following illumination there is more ^{22}Na in the illuminated retinas than in the control retinas. These results were reversed when the retinas remained in the vials 3 or 5 min after illumination before being removed and counted. The illuminated retinas contained less ^{22}Na than the control retinas. The results were inconclusive when the retinas were removed from the vials 2 min after illumination. In Section B of Table I the ratio of the difference between the effluxes occurring in the same retina in the dark and at varying times after illumination to that occurring in the dark are subtracted from those occurring in the control retinas. There is a lower amount of ^{22}Na in the efflux of the illuminated retinas when they were transferred immediately after and 1 min following illumination than in the control retinas, and a greater quantity in the efflux of the control retinas at 3 and 5 min after illumination than in the illuminated retinas. There was no significant

difference in the amounts of ^{22}Na in the efflux of the illuminated and control retinas when the retinas were kept in the second vial for 15 min or longer.

These results show that for a short period of time after illumination, the diffusion of ^{22}Na from the retina is decreased in comparison with the same retina before it was stimulated, or in comparison with a control retina that had not been stimulated. Similar results were reported by BONTING AND BANGHAM[4] for cattle rod outer segments immediately after illumination. They, however, did not report the effect of letting the retinas remain in the vials for several minutes after illumination. In these experiments it is shown that if the retina remained in the vial for at least 3 min after stimulation before transferral, the loss of ^{22}Na from the stimulated retina greatly increased when compared with either the same retina before stimulation, with retinas that remained in the vials for less than 2 min after stimulation, or with control retinas that were not stimulated.

The possibility that after illumination an influx of unlabeled Na^+ causes a dilution of the intraretinal ^{22}Na can explain the decrease in the ^{22}Na in the efflux shortly after illumination has been considered. However, this does not explain the increased ^{22}Na observed in the efflux 3–5 min after illumination.

A more reasonable explanation is as follows: The ^{22}Na leaves the dark-adapted retina in fresh Ringer's solution at a known rate[6]. Since the illuminated retina loses less ^{22}Na immediately after and in the first minute after illumination, and more ^{22}Na 3 min after illumination than a retina that has not been illuminated, it is logical to assume that some additional sodium extrusion process was initiated during this time to enable the efflux of the illuminated retinas to catch up with and then exceed that amount coming from the control retinas. These preliminary results indicate that a sodium pump mechanism has been activated within the retina later than 1 min and less than 3 min after illumination, and within 15 min has restored the retina to the same level as the control retina.

Experiments are currently in progress to determine where this Na^+ pump is located within the retina.

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