J Comp Physiol A (1984) 155:763–770

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology
© Springer-Verlag 1984

# Effects of low sodium, ouabain, and strophanthidin on the shedding of rod outer segment discs

David S. Williams*, Chester Wilson, Ken Linberg, and Steven Fisher
Department of Biological Sciences, University of California, Santa Barbara, California 93106, USA

Accepted August 20, 1984

**Summary.** 1. To test if the light-evoked hyperpolarization of the rods or retinal pigment epithelium (RPE) is important for the light-evoked component of rod disc shedding, eyecups of the anuran, *Xenopus*, were incubated in media that have been reported to have different effects on the membrane potential of these cells.

2. Hyperpolarization was induced by transferral to medium with $Na^+$ entirely replaced by choline$^+$, or mostly replaced by $Li^+$. Shedding in darkness was increased 4-fold in both cases (Figs. 1, 2 and 3).

3. To prevent light-evoked hyperpolarization, eyecups were transferred to medium containing 0.5 mmol/l ouabain. However, light-evoked shedding was not inhibited; instead, it was activated further (Fig. 4). Moreover, ouabain increased shedding in darkness by about 9-fold (Fig. 5).

4. Ouabain likewise activated shedding without light in eyecups of the frog, *Rana* (Fig. 6). In *Rana*, shedding is entirely light-evoked, so that, unlike the case represented by *Xenopus* eyecups, there was no question that inhibition of the light-evoked component could have been masked by overwhelming stimulation of an endogenous component.

5. Strophanthidin, which, in contrast to ouabain, binds reversibly to $Na^+$-$K^+$-ATPase, also activated shedding in *Xenopus* eyecups kept in darkness, even when it was washed out 20 min after light onset (Fig. 7).

6. These results suggest that a particular change in the membrane potential of the rod or RPE cells is not directly responsible for the occurrence of light-evoked rod disc shedding.

7. In addition, the results identify pharmacological treatments that activate shedding in darkness. These treatments should provide a useful tool for future studies of rod disc shedding. It is noted that common effects of these treatments possibly include: (1) inhibition of $Na^+$, $K^+$-ATPase activity, with a resulting increase in ATP; (2) an increase in intracellular $Ca^{2+}$; (3) alteration of melatonin levels.

## Introduction

The shedding of photoreceptor outer segments involves phagocytosis of the terminal outer segment discs by the surrounding retinal pigment epithelium (RPE). As a result, packets of outer segment discs are apparent as phagosomes in the RPE (Young and Bok 1969). Using the number of phagosomes in the RPE as a measure of the extent of shedding, shedding of rod outer segments (ROS) has been shown to increase markedly for a short period following dawn, or 'light onset' (LaVail 1976; Basinger et al. 1976). In lower vertebrates, part, or all of this daily peak in shedding is light-evoked (Basinger et al. 1976). Accordingly, a fundamental question concerning the control of ROS shedding in lower vertebrates follows: which of the physiological changes that result from the onset of light triggers shedding?

Previously published work is compatible with the hypothesis that hyperpolarization of the rods and/or RPE cells, which occurs as a result of light stimulation (Bortoff 1964; Tomita 1970; Steinberg et al. 1970), might be this trigger. Firstly, phagocytosis by macrophages and fibroblasts is accompa-

*Abbreviations:* *ROS* rod outer segment; *RPE* retinal pigment epithelium; *$Na^+$, $K^+$-ATPase* Sodium, potassium-activated adenosine triphosphatase

* Present address: Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024, USA

nied by hyperpolarization of the cells, suggesting that changes in membrane potential might be important for control of some aspect of ingestion (Kouri et al. 1980; Okada et al. 1981). Secondly, recent work by Stowe (1983) has shown that increased extracellular $K^+$ concentration induces shedding of photoreceptor membrane in crab compound eyes not exposed to light at dawn. The normal shedding response in these animals is also mostly light-evoked, and Stowe argued that its inducement by the $K^+$ treatment is probably mediated by depolarization of the photoreceptors (which is their normal response to light). Thirdly, three conditions found to decrease rod or RPE cell membrane potential also inhibit ROS disc shedding: (1) 3-isobutyl-l-methylxanthine (IBMX) depolarizes anuran rods (Lipton et al. 1977), and inhibits their disc shedding (Besharse et al. 1982; Heath and Basinger 1983). (ii) Decreased extracellular $Ca^{2+}$ concentration also depolarizes anuran rods (Brown and Pinto 1974) and inhibits their disc shedding (Greenberger and Besharse 1983). (iii) A decreased bicarbonate concentration suppresses the apical membrane and transepithelial potentials of anuran RPE (Miller and Steinberg 1977a), most components of the light-induced electroretinogram in rats (Winkler et al. 1977), and disc shedding in anurans (Besharse et al. 1980; Heath and Basinger 1983).

We have investigated the possibility that hyperpolarization of the rods or RPE is directly responsible for ROS shedding. As our preparation, we have used anuran eyecups maintained in vitro (Flannery and Fisher 1979; Besharse et al. 1980; Heath and Basinger 1983). Our first experiments were designed to test the effect on ROS shedding of conditions reported to hyperpolarize the rods and RPE cells. Hyperpolarization was induced by substituting choline$^+$ or $Li^+$ for $Na^+$ in the bathing medium (cf. Brown and Pinto 1974). We next tested the effects on ROS shedding of conditions that have been reported to prevent the rod and RPE cells from hyperpolarizing. We exposed eyecups to ouabain or strophanthidin, which rapidly abolish rod hyperpolarizing responses to light (Oakley et al. 1979; Leibovic 1983), and eventually depolarize retinal cells by inhibition of $Na^+$, $K^+$-ATPase activity (Frank and Goldsmith 1967; Steinberg and Miller 1973; Torre 1982).

An important part of our results is the report, for the first time, of pharmacological manipulations that activate shedding in the absence of the onset of light. Some of the findings in the present paper have been published in preliminary form (Williams et al. 1983).

## Materials and methods

The anuran amphibians, *Xenopus laevis*, 2.5–5 cm long, and *Rana pipiens* (northern variety), 5–6.5 cm long, were bought from Nasco (Modesto, CA). They were kept in tanks with Perspex tops and on a 12 h dark/12 h light cycle for at least 3 weeks before being used in experiments. During the light half of the cycle, cold fluorescent lighting provided an illuminance at the water surface of 500 lux. Water temperature was 25–27 °C. The frogs were fed live crickets and Nasco Frog Brittle.

Dissections were carried out under dim red safelights (15 W bulb, with Kodak number 1 or 2 filter). Frogs were decapitated and pithed. Eyes were then either cleanly enucleated or cut out with a small amount of head tissue attached. The anterior half of the eye was removed and the resulting eyecup placed in the standard culture medium.

The standard medium was essentially that used by Heath and Basinger (1983). It contained (mmol/l): 70 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1.25 $KH_2PO_4$, 35 $NaHCO_3$, 10 D-glucose, and 1 g/l casamino acids (containing 13 amino acids and low amounts of vitamins). Its osmolarity was 240 mOsm. All salts were analytical grade from Mallinckrodt, and the casamino acids were from Difco. The experimental media contained: (i) choline chloride and choline bicarbonate instead of NaCl and $NaHCO_3$ (substitution of $Na^+$ by choline$^+$); (ii) LiCl instead of NaCl (substitution of $Na^+$ by $Li^+$); (iii) the addition of 0.5 mmol/l ouabain; or (iv) the addition of 0.5 mmol/l strophanthidin. Ouabain, strophanthidin, and choline bicarbonate were purchased from Sigma, and the choline chloride and LiCl from Mallinckrodt. Media were made the evening before an experiment. Strophanthidin was first dissolved in ethanol; the final concentration of the ethanol was 0.3%, which was also added to the appropriate control solutions. Before use, the media were gassed with humidified 95% $O_2$/5% $CO_2$ in Erlenmeyer flasks for at least 10 min to stabilize the pH at 7.4. Gassing of the media was continued throughout incubation. Temperature was maintained at 26–27 °C by keeping the flasks in a water bath.

Dissections were completed at least 1 h before the onset of light. 20 min before light onset the eyecups were transferred to either fresh standard medium (controls) or an experimental medium. Eyecups that were not to be exposed to light were kept in flasks wrapped in opaque tape. Beginning at the usual time of light onset, eyecups in the non-darkened flasks were exposed to a heat-filtered illuminance of about 800 lux.

2.5 h after light onset, all eyecups were placed in 1.75% glutaraldehyde + 2% formaldehyde in 75 mmol/l sodium cacodylate buffer (pH 7.4), and fixed for 3–24 h. They were postfixed in 1% $OsO_4$ in the same buffer, dehydrated in ethanol, and embedded in Araldite. Blocks were aligned so that sections (1 μm thick) were cut along the long axes of the rods and the dorso-ventral axis of the eyecup, near the optic nerve. After staining the sections with Azure II, the extent of shedding was quantified under a light microscope by counting the number of phagosomes, greater than 2 μm in their greatest diameter, in the RPE of one well-aligned, complete section of each eyecup (i.e., 3–5 mm of *Xenopus* RPE and 5–8 mm of *Rana* RPE) (Fig. 1). Phagosomes were distinguished from myeloid bodies according to their stain intensity and shape. For each *Xenopus* eye, phagosome counts were converted to an average number of phagosomes/mm, since the spacing of the rods is fairly uniform throughout the retina. Shedding of *Rana* rods was expressed as a percentage: the number of phagosomes/100 ROSs in the retinal expanse counted. In both cases, the possibility of a contribution to phagosome numbers by the cones was ignored. The COSs are very much smaller than the ROSs, and their phagosomes appear to be mostly less than 2 μm in diame-

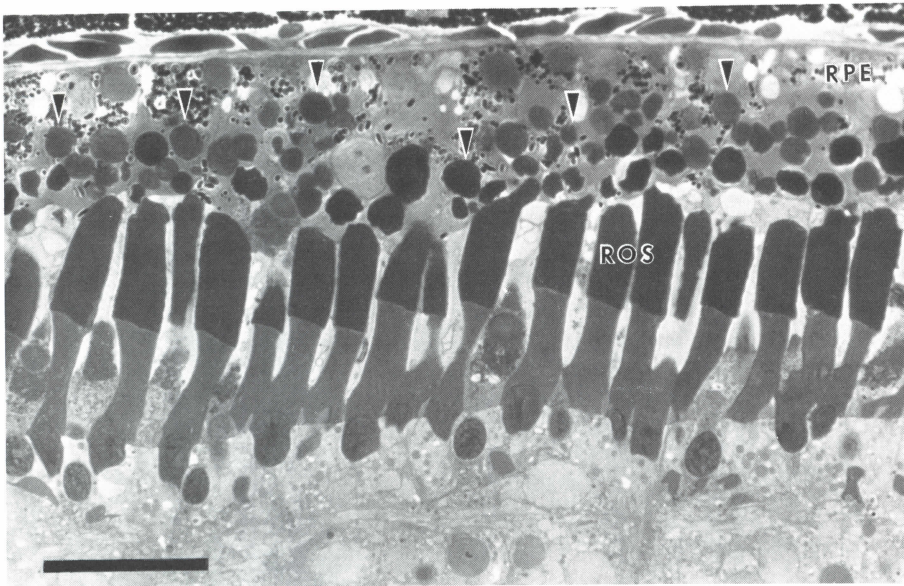


**Fig. 1.** Light micrograph illustrating rod outer segment (*ROS*) shedding in an eyecup of *Xenopus* that was transferred to medium with $Na^+$ replaced by $choline^+$ and kept in darkness. Numerous phagosomes (e.g. arrows) are apparent in the retinal pigment epithelium (*RPE*), indicating extensive shedding. In the particular region shown, it is evident that each ROS has provided more than one phagosome. ROSs are consequently quite short: their length is 15–20 μm, compared to an average length of 45 μm for ROSs in standard medium. Scale bar = 25 μm

ter. Where required, a one-tailed *t*-test was employed to determine the probability of no significant difference between experimental and control samples.

## Results

### *ROS shedding in standard medium*

The number of phagosomes from shed ROS discs is greatest about 2 h after light onset (LaVail 1976; Besharse et al. 1977). In *Xenopus*, we found the amount of ROS shedding 1 h before the usual time of light onset (i.e. basal shedding) to be $12.1 \pm 1.2$ (mean $\pm$ SEM; $n=3$) phagosomes/mm RPE. 2.5 h after the usual time of light onset, the amount of shedding was $18.1 \pm 1.4$ ($n=39$) phagosomes/mm RPE in darkness, and $30.8 \pm 4.5$ ($n=14$) phagosomes/mm RPE when exposed to light (overall means of all experiments). These latter values, less the amount of basal shedding represent, respectively, the shedding response elicited by the endogenous component alone, and the shedding response elicited by the endogenous and light-evoked components combined. Our values are similar to those found under the same lighting conditions in *Xenopus* tadpoles (Besharse et al. 1977) and adults (Williams, unpublished) in vivo, and in adult *Xenopus* eyecups, maintained in Wolf and Quimby amphibian culture medium with 35 mmol/l $HCO_3^-$ (Besharse et al. 1980); although we found a slightly higher amount of basal shedding, and shedding in darkness, and thus a somewhat smaller light-evoked component of the shedding peak.

The basal ROS shedding in *Rana* was $1.4 \pm 1.0$ ($n=3$) phagosomes/100 ROSs (i.e. 1.4%). 2.5 h after the normal time of light onset, the amount of shedding in darkness was $1.1 \pm 0.3$ ($n=4$) phagosomes/100 ROSs, and after exposure to light, $10.0 \pm 1.4$ ($n=4$) phagosomes/100 ROSs (Fig. 6). Heath and Basinger (1983) found that the amount of ROS shedding in *Rana,* held in constant light for 2 days and then exposed to 60 min darkness followed by 90 min light, was similar in vitro (using practically the same culture medium as we did) and in vivo (about 20%). Our finding of 10% shedding after 2.5 h light exposure is considerably less than the daily maximum of 20–25% 'fresh' phagosomes found in vivo 1–1.5 h after light onset by Basinger et al. (1976) and Basinger and Hollyfield (1980). However, because of differences in timing and defining phagosomes, this comparison has limited value. Since the amount of shedding in darkness, 2.5 h after the time of light onset, was no greater than the basal amount, our in vitro measurements agree with the in vivo findings of Basinger and coworkers (Basinger et al. 1976; Basinger and Hollyfield 1980), that the daily peak of shedding in *Rana* has no endogenous component: it is entirely light-evoked.

### *Effect of $Na^+$ substitution with $choline^+$ or $Li^+$: Xenopus*

Replacement of external $Na^+$ by $choline^+$ or $Li^+$ (or sucrose) causes rods in isolated toad retinae to hyperpolarize by about 30 mV (Brown and Pinto 1974). We examined the effect of replacing $Na^+$ entirely with $choline^+$ or partially by $Li^+$ on ROS shedding in darkness. These treatments re-

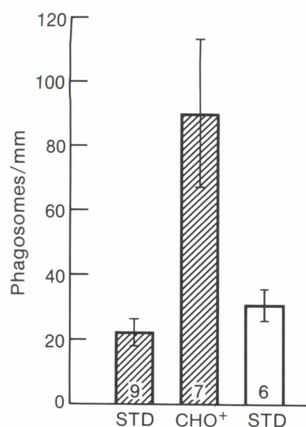


**Fig. 2.** Effect of replacing of medium $Na^+$ entirely by choline$^+$ ($CHO^+$) on ROS shedding in *Xenopus* eyecups. The extent of shedding was measured by the number of phagosomes/mm of RPE. STD represents the amount of shedding in standard medium in the same experiments. Shading indicates no exposure to light. The number of eyecups examined for each condition is shown at the base of each bar. Data were aggregated from 2 separate experiments, and are represented by the mean $\pm 1$ standard error of the mean

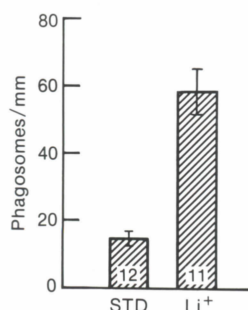


**Fig. 3.** Effect of partial replacement of medium $Na^+$ by $Li^+$ on ROS shedding in *Xenopus* eyecups. STD, standard medium with 105 mmol/l $Na^+$; $Li^+$, medium containing 70 mmol/l $Li^+$ and 35 mmol/l $Na^+$. Eyecups were maintained in darkness. Each bar represents the mean ($\pm 1$ SEM; *n* shown at base). Data were aggregated from 3 experiments

sulted in a 4-fold increase in shedding (Figs. 1, 2 and 3). In fact, shedding was 3-fold greater following $Na^+$ substitution in darkness than after exposure to light in the control medium (Fig. 2).

*Effect of ouabain: Xenopus*

By inhibition of $Na^+$, $K^+$-ATPase activity, ouabain equilibrates the $Na^+$ and $K^+$ ion gradients across the rod and RPE cell membranes, and the cells eventually depolarize (Frank and Goldsmith 1967; Steinberg and Miller 1973; Torre 1982). Rods appear to maintain some negative membrane potential, possibly due to impermeable anions (Bastian and Fain 1982), but this potential is small (Woodruff et al. 1982). Hyperpolarizing responses

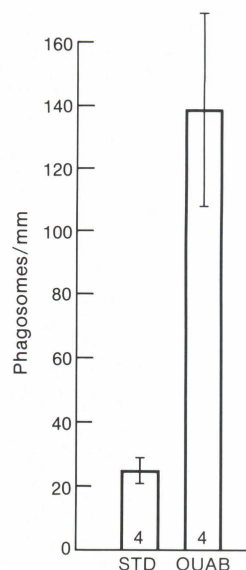


**Fig. 4.** Comparison of ROS shedding in *Xenopus* eyecups in standard medium (STD) with ROS shedding in medium that has had 0.5 mmol/l ouabain added (OUAB). Eyecups in both media were exposed to light for 2.5 h from the usual time of light onset. Data are represented by the mean ($\pm 1$ SEM) of 4 eyecups from 1 experiment

to light are rapidly abolished by ouabain (Oakley et al. 1979; Leibovic 1983).

If hyperpolarization of the rods and RPE cells is directly responsible for light-evoked ROS shedding, shedding in response to light should be inhibited by ouabain. It can be seen clearly in Fig. 4, however, that shedding is very much stimulated. The addition of 0.5 mmol/l ouabain results in a 5.6-fold increase in the number of phagosomes.

On examining the effect of ouabain in darkness, a similar activation of shedding was found. Phagosomes were 8.6 times more abundant in the presence of 0.5 mmol/l ouabain (Fig. 5). Ouabain, therefore, not only fails to inhibit shedding, but strongly induces it.

*Effect of ouabain: Rana*

If the mechanisms regulating the light-evoked and endogenous components of shedding operate independently, it follows that a drug could still inhibit the light-evoked component, but at the same time effect overall activation because of its overwhelming activation of the endogenous component. The above experiments, showing the activation of shedding by ouabain in *Xenopus*, leave open this possibility. However, as mentioned above, shedding in *Rana* has no endogenous component of activation (first section of Results; Basinger et al. 1976; Basinger and Hollyfield 1980). We therefore tested the

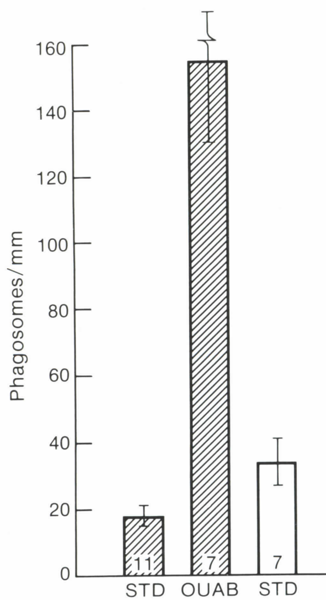


**Fig. 5.** Effect of 0.5 mmol/l ouabain (OUAB) on ROS shedding in *Xenopus* eyecups in darkness. STD represents the amount of shedding in standard solutions in darkness (shaded) or light (unshaded) in the same experiments. Data were aggregated from 3 separate experiments, and are represented by the mean (±1 SEM; *n* shown at the base of each bar)

effects of 0.5 mmol/l ouabain on *Rana* eyecups. It is clear from Fig. 6 that shedding is also stimulated by ouabain in *Rana*.

### *Effect of strophanthidin: Xenopus*

The inhibition of $Na^+$, $K^+$-ATPase by ouabain is only very slowly reversible (Hoffman 1969; Dunham and Hoffman 1971), so that it was not possible to test the effect of a short exposure to ouabain. This is important to the extent that long exposures to ouabain may result in changes subsequent to depolarization (e.g. Gorman and Marmor 1974). In contrast to inhibition by ouabain, inhibition of $Na^+$, $K^+$-ATPase by strophanthidin is reversible and can be removed by washing out the drug with fresh standard medium (Hoffman 1966; Sachs 1974). In darkness, 0.1 mmol/l strophanthidin depolarizes toad rods by about 5 mV (Torre 1982).

We examined the response of eyecups kept in darkness and exposed to medium containing 0.5 mmol/l strophanthidin (1) for the entire post-light onset period, as above for ouabain, and (2) until 20 min after the time of light onset. Because eyecups were transferred to experimental media 20 min before the time of light onset, they were exposed to strophanthidin for a total of 40 min in the second procedure. After exposure, they were washed several times with fresh standard medium.

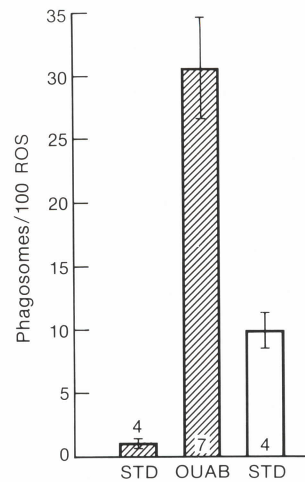


**Fig. 6.** Effect of 0.5 mmol/l ouabain (OUAB) on ROS shedding in *Rana* eyecups in darkness. STD represents the amount of shedding in standard solutions in darkness (shaded) or light (unshaded) in the same experiments. Each bar represents the mean (±1 SEM; *n* shown at base). Data were aggregated from 2 separate experiments

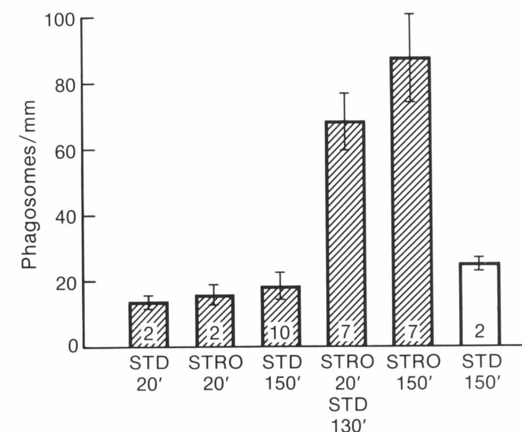


**Fig. 7.** Effect of 0.5 mmol/l strophanthidin (STRO) on ROS shedding in *Xenopus* eyecups in darkness. The 2 bars on the left represent the amount of shedding in standard medium (STD) and in the presence of strophanthidin 20 min after the usual time of light onset. The other 4 bars represent the amount of shedding 2.5 h after the usual time of light onset in (from left to right): standard medium, medium that contained strophanthidin until 20 min after light onset when it was replaced by standard medium; medium with strophanthidin; standard medium. Only the last was exposed to light. Unlike in the other experiments of the present report, all media contained 0.3% ethanol. Each bar represents the mean (±SEM; *n* shown at the base). Data were from 1 experiment, except for the 3rd, 4th, and 5th bars which include data from 2 further experiments

How long the rods and RPE cells were fully exposed to 0.5 mmol/l strophanthidin is unknown, but since a significant penetration time is likely, it was probably somewhat less than 40 min. Because of the rapid reversibility of strophanthidin, exposure to lower concentrations would have been less than 80 min.

The effect of 0.5 mmol/l strophanthidin on ROS shedding is illustrated in Fig. 7. 20 min after the time of light onset, shedding, in both standard medium and medium with strophanthidin, was not significantly greater than the basal level of shedding ($P > 0.1$ in both cases). However, 2.5 h after the time of light onset, in comparison to eyecups kept in standard medium, shedding was 4.5-fold greater in eyecups that had been exposed to strophanthidin for the entire post-light onset period, and 3.4-fold greater in eyecups exposed to strophanthidin until 20 min after light onset, followed by 130 min in standard medium. Exposure to $10^{-5}$ mol/l strophanthidin from 20 min before until 20 min after the time of light onset elicited a shedding response similar to that following exposure to 0.5 mmol/l strophanthidin over the same interval: $55.0 \pm 17.3$ ($n=3$) phagosomes/mm, compared to $58.6 \pm 9.7$ ($n=4$) phagosomes/mm for 0.5 mmol/l strophanthidin and $13.4 \pm 5.1$ ($n=5$) for standard medium in the same experiment. Consequently, it seems that a short inhibition of $Na^+$, $K^+$-ATPase activity at the time of light onset is sufficient to activate a shedding response which is not manifest until later.

## Discussion

### *Membrane potential*

ROS shedding was activated by pharmacological conditions that have been reported to hyperpolarize the rod and RPE cells, and by conditions reported to prevent light-evoked hyperpolarization and, indeed, eventually cause depolarization of these cells. These reports have mostly involved isolated retinae (Brown and Pinto 1974; Oakley et al. 1979; Torre 1982) or isolated choroid-RPE (Steinberg and Miller 1973). In an eyecup preparation, as used in the present study, the photoreceptors and RPE cells are less readily exposed to alterations of the bathing medium. Nevertheless, Frank and Goldsmith (1967) showed that the electroretinogram response of frog retinae was diminished after 6 min, and completely abolished after 40 min when the retinae were exposed to 0.1 mmol/l ouabain on the vitreal side only. And Cervetto (1973) showed that the cones in a turtle eyecup hyperpolarized within 5 min after $Na^+$ in the bathing medium was replaced by choline$^+$. Moreover, since all of our treatments activated ROS shedding, we know that they all penetrated the eyecup. The site(s) of action in the eyecup is undetermined. But because $Na^+$ substitution by choline$^+$ or $Li^+$, and the addition of ouabain or strophanthidin are likely to have opposite effects on the membrane potential of all cells of the neural retina and RPE, our results indicate that they activated shedding by some means other than by a particular change in membrane potential. In conclusion, our results suggest that a particular change in the membrane potential of the rods or RPE is not directly responsible for the occurrence of light-evoked ROS shedding.

### *Site of activation by ouabain*

As noted above, we do not know where in the eyecup the stimulating conditions exert their effect. It appears, however, that we can rule out the possibility of ouabain stimulation by direct action on the RPE. The apical surface of the RPE does have binding sites for ouabain (Bok 1982), but Masterson and Chader (1981) found that phagocytosis of bovine ROSs by cultured chick RPE was somewhat *inhibited*, rather than stimulated, by 0.1 mmol/l ouabain.

### *Mechanisms of activation by low $Na^+$, ouabain, and strophanthidin*

The mechanisms by which low $Na^+$, and ouabain and strophanthidin activated ROS shedding are obscure. However, it is noteworthy that these treatments should produce some common effects.

Firstly, they should all inhibit $Na^+$, $K^+$-ATPase activity: low $Na^+$ decreasing the intracellular $Na^+$, and, in turn, the extracellular $K^+$ that is available for transport; ouabain and strophanthidin by binding to an external site of the enzyme (Trachtenberg et al. 1981). This inhibition should reduce ATP utilization (Winkler 1981), and thus possibly increase the availability of ATP for other cellular processes. Because of a decrease in internal rod $Na^+$ and subretinal space $K^+$, the onset of light also inhibits the $Na^+$, $K^+$-ATPase activity of the rods and RPE cells (Oakley 1983). The concentration of ROS ATP has been shown to increase in response to light or ouabain (Kimble et al. 1980).

Secondly, these treatments should effect a drop in the extracellular to intracellular $Na^+$ gradient. Many cells, including photoreceptors (Schnetkamp 1980; Bastian and Fain 1982) and possibly RPE cells (Miller and Steinberg 1977b), appear to possess $Na^+/Ca^{2+}$ exchange mechanisms that are driven by this $Na^+$ gradient (review: Blaustein 1974). Consequently, ouabain, strophanthidin, and low $Na^+$ would result in elevated intracellular $Ca^{2+}$ levels.

Thirdly, indirect evidence suggests that ouabain, strophanthidin and low $Na^+$ might alter levels of melatonin in the retina. This possibility, however tenuous, warrants consideration because melatonin has been shown to inhibit the phagocytic ability of cultured RPE cells (Ogino et al. 1983) and affect ROS shedding in *Xenopus* eyecups (Besharse and Dunis 1983). Ouabain inhibits cyclic AMP stimulation of N-acetyltransferase (NAT) activity in the pineal (Parfitt et al. 1975), and inhibits serotonin uptake in the retina (Thomas and Redburn 1979). The rate of serotonin uptake by the retina is proportional to the external concentration of $Na^+$ (Osborne 1982), so that lowered $Na^+$ also inhibits serotonin uptake. Melatonin is ultimately synthesized from serotonin, with NAT the rate-limiting enzyme in its production.

The present report provides the first account of pharmacological activation of outer segment shedding in the absence of light. Investigation of the common effects of the activating treatments in relation to ROS shedding should provide a useful starting point for furthering our understanding of the mechanisms by which ROS shedding is regulated.

*Acknowledgements.* We are grateful to Sally Stowe for correspondence about her work with *Leptograpsus* prior to publication, and to Debbie Mustard and Melanie Fujii for unusually swift and accurate typing. This work was supported by NIH grants EY04670 and EY00888.

## References

Basinger SF, Hollyfield JG (1980) Control of rod shedding in the frog retina. Neurochem 1:81–92

Basinger S, Hoffman R, Matthes M (1976) Photoreceptor shedding is initiated by light in the frog retina. Science 194:1074–1076

Bastian BL, Fain GL (1982) The effects of sodium replacement on the responses of toad rods. J Physiol (Lond) 330:331–347

Besharse JC, Dunis DA (1983) Methoxyindoles and photoreceptor metabolism: activation of rod shedding. Science 219:1341–1343

Besharse JC, Hollyfield JG, Rayborn MI (1977) Turnover of rod photoreceptor outer segments. II. Membrane addition and loss in relationship to light. J Cell Biol 75:507–527

Besharse JC, Terrill RO, Dunis DA (1980) Light-evoked disc shedding by rod photoreceptors in vitro: relationship to medium bicarbonate concentration. Invest Ophthalmol Visual Sci 19:1512–1517

Besharse JC, Dunis DA, Burnside B (1982) Effects of cyclic adenosine 3′,5′-monophosphate on photoreceptor disc shedding and retinomotor movement. J Gen Physiol 79:775–790

Blaustein MP (1974) The interrelationship between sodium and calcium fluxes across cell membrane. Rev Physiol Biochem Pharmacol 70:33–74

Bok D (1982) Autoradiographic studies on the polarity of plasma membrane receptors in retinal pigment epithelium. In: Hollyfield JG (ed) The structure of the eye. Elsevier, New York, pp 247–256

Bortoff A (1964) Localization of slow potential responses in the *Necturus* retina. Vision Res 4:627–636

Brown JE, Pinto LH (1974) Ionic mechanisms for the photoreceptor potential of the retina of *Bufo marinus*. J Physiol (Lond) 236:575–591

Cervetto L (1973) Influence of sodium, potassium and chloride ions on the intracellular responses of turtle photoreceptors. Nature 241:401–403

Dunham PB, Hoffman JF (1971) Active cation transport and ouabain binding in high potassium and low potassium red blood cells of sheep. J Gen Physiol 58:94–116

Flannery JG, Fisher SK (1979) Light-triggered rod disc shedding in *Xenopus* retina in vitro. Invest Ophthalmol Visual Sci 18:638–642

Frank RN, Goldsmith TH (1967) Effects of cardiac glycosides on electrical activity in the isolated retina of the frog. J Gen Physiol 50:1585–1606

Gorman ALF, Marmor MF (1974) Long-term effect of ouabain and sodium pump inhibition on a neuronal membrane. J Physiol (Lond) 242:59–60

Greenberger LM, Besharse JC (1983) Photoreceptor disc shedding in eye cups: inhibition by deletion of extracellular divalent cations. Invest Opthalmol Visual Sci 24:1456–1464

Heath AR, Basinger SF (1983) Frog rod outer segment shedding in vitro: histologic and electrophysiologic observations. Invest Ophthalmol Visual Sci 24:277–284

Hoffman JF (1966) The red cell membrane and the transport of sodium and potassium. Am J Med 41:666–680

Hoffman JF (1969) The interaction between tritiated ouabain and the Na-K pump in red blood cells. J Gen Physiol 54:3435–3505

Kimble EA, Svobody RA, Ostroy SE (1980) Oxygen consumption and ATP changes of the vertebrate photoreceptor. Exp Eye Res 31:271–288

Kouri J, Noa M, Diaz B, Niubo E (1980) Hyperpolarization of rat peritoneal macrophages phagocytosing latex particles. Nature 283:868–870

LaVail MM (1976) Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. Science 194:1071–1074

Leibovic KN (1983) *Bufo* rod responses reveal a contribution of the sodium pump. Soc Neurosci Abstr 9:164

Lipton SA, Ostroy SE, Dowling JE (1977) Electrical and adaptive properties of rod photoreceptors in *Bufo marinus*. I. Effects of altered extracellular $Ca^{2+}$ levels. J Gen Physiol 70:747–770

Masterson E, Chader GJ (1981) Pigment epithelial cells in culture: metabolic pathways required for phagocytosis. Invest Ophthalmol Visual Sci 20:1–7

Miller SS, Steinberg RH (1977a) Passive ionic properties of frog retinal pigment epithelium. J Membr Biol 36:337–372

Miller SS, Steinberg RH (1977b) Active transport of ions across frog retinal pigment epithelium. Exp Eye Res 25:235–248

Oakley B (1983) Potassium homeostasis in the toad retina. Invest Ophthalmol Visual Sci 23 (Suppl):179

Oakley B, Flaming DG, Brown KT (1979) Effects of rod receptor potential upon retinal extracellular potassium concentration. J Gen Physiol 74:713–737

Ogino N, Matsumura M, Shirakawa H, Tsukuhara I (1983) Phagocytic activity of cultured retinal pigment epithelial cells from chick embryo; inhibition by melatonin and cyclic AMP, and its reversal by taurine and cyclic GMP. Ophthalmic Res 15:72–89

Okada Y, Tsuchiya W, Yada T, Yano J, Yawo H (1981) Phagocytic activity and hyperpolarizing responses in L-strain mouse fibroblasts. J Physiol (Lond) 313:101–119

Osborne NN (1982) Uptake, localization, and release of serotonin in the chick retina. J Physiol (Lond) 331:469–479
Parfitt A, Weller JL, Klein DC, Sakai KK, Marks BH (1975) Blockade by ouabain or elevated potassium ion concentration of the adrenergic and adenosine cyclic 3′,5′-monophosphate-induced stimulation of pineal serotonin N-acetyltransferase activity. Mol Pharmacol 11:241–255
Sachs JR (1974) Interaction of external K, Na, and cardioactive steroids with the Na-K pump of the human red blood cell. J Gen Physiol 63:123–143
Schnetkamp PPM (1980) Ion selectivity of the cation transport system of isolated intact cattle rod outer segments. Biochim Biophys Acta 598:66–90
Steinberg RH, Miller SS (1973) Aspects of electrolyte transport in frog pigment epithelium. Exp Eye Res 16:365–372
Steinberg RH, Schmidt R, Brown KT (1970) Intracellular responses to light from cat pigment epithelium: origin of the electroretinogram c-wave. Nature 227:728–730
Stowe S (1983) Light-induced and spontaneous breakdown of the rhabdoms in a crab at dawn; depolarization versus calcium levels. J Comp Physiol 153:365–375
Thomas TN, Redburn DA (1979) 5-hydroxytryptamine – a neurotransmitter of bovine retina. Exp Eye Res 28:55–61
Tomita T (1970) Electrical activity of vertebrate photoreceptors. Q Rev Biophys 3:179–222
Torre V (1982) The contribution of the electrogenic sodium-potassium pump to the electrical activity of toad rods. J Physiol (Lond) 333:315–341
Trachtenberg MC, Packey DJ, Sweeney T (1981) In vivo functioning of the $Na^+$, $K^+$-activated ATPase. In: Horecker BL, Stadtman ER (eds) Current topics in cellular regulation. Academic Press, New York, vol 19, pp 159–217
Williams DS, Wilson C, Fisher SK (1983) Activation of rod outer segment shedding: the unimportance of membrane potential. Soc Neurosci Abstr 9:503
Winkler BS (1981) Glycolytic and oxidative metabolism in relation to retinal function. J Gen Physiol 77:667–692
Winkler BS, Simson V, Benner J (1977) Importance of bicarbonate in retinal function. Invest Ophthalmol Visual Sci 16:766–768
Woodruff ML, Fain GL, Bastian BL (1982) Light-dependent ion influx into toad photoreceptors. J Gen Physiol 80:517–536
Young RW, Bok D (1969) Participation of the retinal pigment epithelium in the rod outer segment renewal process. J Cell Biol 42:392–403