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Light-stimulated protein movement in rod photoreceptor cells of the rat retina

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We examined the intracellular distribution of three proteins involved in the cyclic GMP cascade of visual transduction; cGMP phosphodiesterase, the α-subunit of G-protein and arrestin. In adult rats, light-induced changes in the amounts of G and arrestin in the photoreceptor cell outer segments were observed both by polyacrylamide gel analysis of purified ROS and by immunocytochemical localization on retinal sections. In dark conditions, G was concentrated in the outer segments of photoreceptor cells while in the light G_α was seen in the inner segments and the outer nuclear layer. Arrestin had the opposite distribution, appearing in the inner segments and outer nuclear layer under dark conditions and in the ROS under light conditions. In contrast, PDE, the enzyme which is activated by G and inhibited by arrestin showed no light-stimulated movement. In both light- and dark-adapted retinas, PDE was localized primarily in the outer segments of the photoreceptor cells.

Photoreceptor; Phosphodiesterase; Arrestin; G-binding protein; protein movement

1. INTRODUCTION

The photoreceptor cells of the retina are morphologically compartmentalized cells having inner and outer segment regions which are connected by a narrow cilium. The mitochondria and biosynthetic machinery of the photoreceptor cells are localized in the inner segment. The outer segment, which consists of stacks of membranous disks surrounded by a plasma membrane, houses all of the components necessary for visual transduction. Thus, protein components of the cGMP cascade of visual transduction are synthesized in the inner segment and must be transported across the cilium to the outer segment. While much work has been done on the synthesis, transport and incorporation of rhodopsin into forming disk membranes of the outer segment [1], little is known about how soluble and peripheral membrane proteins are transported between the inner and outer segments. In a recent paper [2], it was shown that G_α, a peripheral membrane protein that mediates light-stimulated activation of PDE [3], is synthesized in the morning but is not localized in the outer segment until the night. The results of these experiments would suggest that light, rather than synthesis, regulates the distribution of proteins within photoreceptor cells. Immunocytochemical studies have shown that arrestin, a protein thought to quench light-activated PDE activity [4,5], is localized to the ROS in the light, while in the dark it is found in the inner segments and outer nuclear layer [6]. Since both G_α and arrestin have differen-

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Abbreviations: ROS, rod outer segments; PDE, cGMP phosphodiesterase; G_α, α-subunit of G-protein; arrestin, 48 kDa protein and S-antigen; PBS, phosphate-buffered saline; BSA, bovine serum albumin

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tial membrane affinities in light and dark, the immunocytochemical observations may have been the result of antigenic masking and unmasking rather than, or in addition to, the translocation of the proteins between intracellular compartments. In the present report, we have examined the localization of PDE, G_α and arrestin in light- and dark-adapted retinas using both SDS-PAGE analysis of purified ROS and light microscopic immunocytochemical techniques. We demonstrate that within the photoreceptor cells there are light-induced movements of the G_α and arrestin, but not of PDE.

2. MATERIALS AND METHODS

2.1. *Animals*

Adult male Wistar rats (150–200 g) were purchased from Charles Rivers Laboratory. The animals were maintained on a 12 h light/dark cycle for at least two weeks prior to being used in the experiments. In the dark experiments, all procedures were carried out under dim red light. Rats were light-adapted under normal room light (250 lux at cage level). Animals for all studies were killed by CO_2 inhalation and the eyes were immediately removed. The eyes were either fixed for immunohistochemistry or the retinas removed for the isolation of ROS.

2.2. *SDS-PAGE*

Intact ROS were prepared from retinas isolated from light- and dark-adapted rats using the method of Schnetkamp [7]. Proteins were separated on 10% SDS-PAGE microslab gels using the buffer system of Laemmli [8]. Gels were run at a constant voltage of 150 V then fixed and stained in 0.25% Coomassie brilliant blue R in 50% methanol, 10% acetic acid and destained by diffusion in 5% methanol, 10% acetic acid and destained by diffusion in 5% methanol, 10% acetic acid. Gels were scanned on a Canalco model K densitometer (Rockville, MD) and areas under the peaks were quantitated using a Numonics integrator (Numonics Corp, Landsdale, PA).

2.3. *Immunological procedures*

ROS proteins separated on SDS-polyacrylamide gels were electrophoretically transferred to nitrocellulose paper at 100 V for 1 h using a Mini-Transblot apparatus (Bio-Rad Laboratories, Richmond, CA). The nitrocellulose paper was washed for 1 h in 0.05 M Tris, pH 8.0, 0.15 M NaCl, 0.05% Nonidet P-40 and 0.1% BSA to block non-specific binding sites. The blots were incubated in the same buffer with polyclonal antisera to arrestin (gift of Dr Igal Gery), the α-subunit of G-protein (gift of Dr A. Spiegel) and PDE (produced in rabbits in our laboratory to PDE purified by Dr A. Sitaramayya). All primary antisera were used at a dilution of 1:500 and were incubated with the blots for 1 h. After thorough washing a secondary antibody of peroxidase-labeled goat anti-rabbit IgG (Cappel, Malvern, PA) was diluted to 1:5000 in the same blocking buffer and applied for 45 min. Bands with absorbed antibodies were visualized with diaminobenzidine (Polysciences, Warrington, PA), 0.5 mg/ml, in 0.05 M Tris, pH 7.6, containing 0.0030% hydrogen peroxide.

Eyes from light- and dark-adapted adult Wistar rats were fixed in 2% paraformaldehyde, 0.1% glutaraldehyde, 3% sucrose, 1 mM $MgCl_2$ in PBS buffer, pH 7.4, for 1 h at room temperature. At the time of enucleation, a small incision was made in the cornea to allow for more rapid fixation of the retina. Following fixation, the anterior portions of the eyes were removed and the eyecups were washed for 1 h with several changes of PBS. The eyecups were placed in PBS/sodium borohydride (0.5 mg/ml) for 30 min at 4°C then equilibrated in PBS/30% sucrose overnight. The eyecups were embedded in Tissue Tek II (Lab Tek, Naperville, IL) and frozen in liquid nitrogen. Six micrometer cryostat sections were cut and mounted on gelatin-coated coverslips. Sections were air dried for 30 min and then were fixed again for 2 min with 2% paraformaldehyde, 3% sucrose and 1 mM $MgCl_2$ in PBS. Sections were rinsed with PBS and non-specific sites were blocked using 1% BSA and 0.05% Tween 20 in PBS, pH 7.4. Primary antibodies were diluted in the same buffer at 1:50 for anti-PDE and anti-G_α and 1:100 for anti-arrestin. Sections were incubated with the primary antibodies for 1 h at room temperature in a humidified chamber. Unbound antibody was removed by 5 washes with the blocking buffer. Sections were then incubated with rhodamine-conjugated goat anti-rabbit $F(ab)_2$ (Jackson Laboratories, Avondale, PA) at a dilution of 1:150, washed again and mounted onto slides.

Control tissue sections were incubated either with preimmune rabbit serum followed by secondary antibody or with secondary antibody alone. Slides were examined and photographed on a Zeiss Research microscope equipped with a 63 × Planapo phase objective with a N.A> of 1.4. Photographs were taken with Kodak Tri-X film. For each experimental condition, $n = 4$ or greater.

3. RESULTS AND DISCUSSION

The protein composition of ROS isolated from light- and dark-adapted rats is shown in fig.1. Protein samples were run in duplicate on the same gel and half of the gel was stained for protein with Coomassie blue and the other half was transferred to nitrocellulose paper for immunoblot analysis. It is evident on the Coomassie-stained gel that there were differences in the amounts of arrestin and G_α present in the outer segments prepared from dark- and light-adapted animals. In the dark-adapted ROS, there was a greater amount of the G_α than in light-adapted ROS. The reverse pattern was seen with arrestin; higher levels were observed in light-adapted ROS preparations than in dark-adapted ROS. These results were confirmed by immunoblot analysis (fig.1). The same nitrocellulose blot was cut horizontally between the PDE and arrestin bands. The upper half of the blot was incubated with antibody to PDE, while the lower half was sequentially incubated with antibodies to arrestin and G_α. Combining the upper and lower halves of the immunoblots facilitates comparison of the amount of immunoreactive protein in one lane as well as between light and dark samples. The results of the immunoblots confirm the results seen on the Coomassie-stained gels, demonstrating that the amounts of arrestin and G_α in the outer segments change as a function of light exposure. These differences do not appear to be due to net changes in synthesis and degradation of these proteins since light/dark changes were not observed when total retinal protein samples were run on SDS-PAGE and immunoblotted with the same antibodies.

The rate at which arrestin moves from the inner to the outer segment in response to light exposure was determined by sacrificing rats at various times after the onset of light, isolating the ROS and separating the proteins on SDS-PAGE. The gels

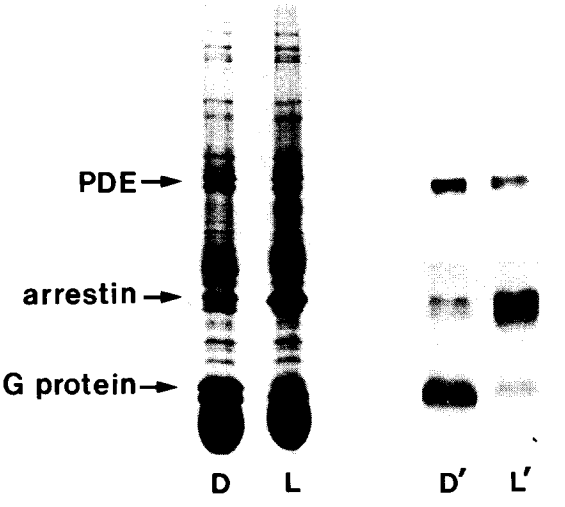

Fig.1. SDS-polyacrylamide gel electrophoresis analysis of rat rod outer segments prepared from dark(D)- and light(L)-adapted animals. The corresponding immunoblot, D' and L', stained with antibodies to PDE, arrestin and the α-subunit of G protein. Note the light-dark changes in arrestin and G_α in the ROS isolated from light- and dark-adapted animals.

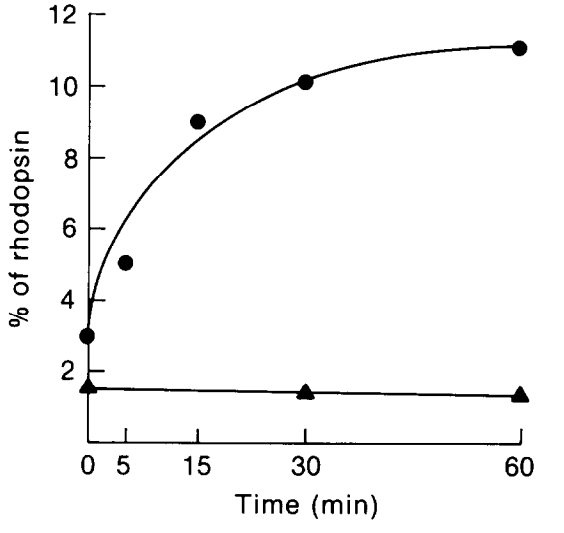

Fig.2. Rat rod outer segments were prepared in the dark and 5, 15, 30 and 60 min after light onset. The purified ROS were run on SDS-polyacrylamide gels. Gels were scanned and the areas under PDE (▲), arrestin (●) and opsin peaks were quantified by densitometry. The relative amounts of arrestin and PDE are expressed as a percentage of opsin on the gel.

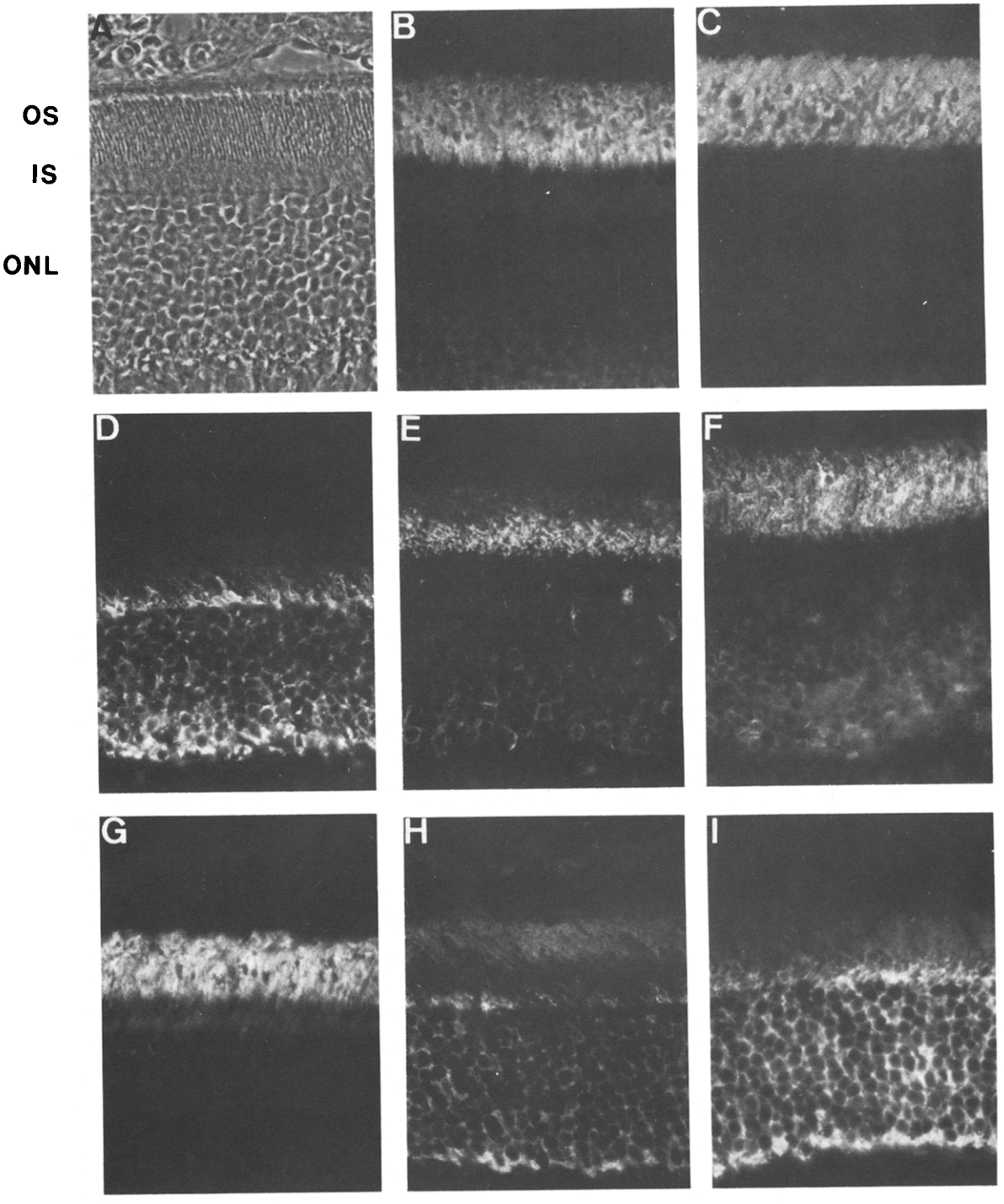

Fig.3. Indirect immunofluorescence localization with antibodies to PDE, arrestin and G_α. (A) The regions of the outer segments (OS), inner segments (IS) and the outer nuclear layer (ONL) are indicated. PDE localization in dark(B)- and light(C)-adapted rat retinas. Arrestin localization in animals dark-adapted for 12 h (D), after 15 min (E) and 60 min (F) of light. G_α localization in dark-adapted retinas (G), 15 min (H) and 60 min of light (I).

were scanned and the amounts of PDE and arrestin relative to rhodopsin were determined. The light-induced increase of arrestin in the outer segments occurred with a half-time of about 10 min (fig.2). No significant light-induced changes were observed in the amount of PDE present in the outer segment.

The subcellular distributions of PDE, arrestin and G_α were examined in rat retinas fixed before and after the onset of light. The antibody to PDE was shown to react primarily with the outer segment region of the photoreceptor cells both in the light and in the dark (fig.3B,C). Arrestin was found in the inner segments and the inner nuclear layer in retinas prepared from dark-adapted animals (fig.3D). After 15 min in room light, arrestin was localized in the basal region of the outer segments, but the labeling did not extend to the tips of the outer segments (fig.3E). After 1 h of light, anti-arrestin labeling was seen throughout the outer segments.

Tissue sections reacted with antibody to G_α demonstrated that the outer segments are most heavily labeled in the dark while the inner segments and the outer nuclear layer are most heavily labeled in the light (fig.3H and I). Similar results were presented in a recent communication by Brann and Cohen [2].

To determine whether the movement of arrestin and G_α was light-stimulated or circadian, the following paradigm was used: rats maintained on a regular, 12 h light/dark cycle received 3 h of

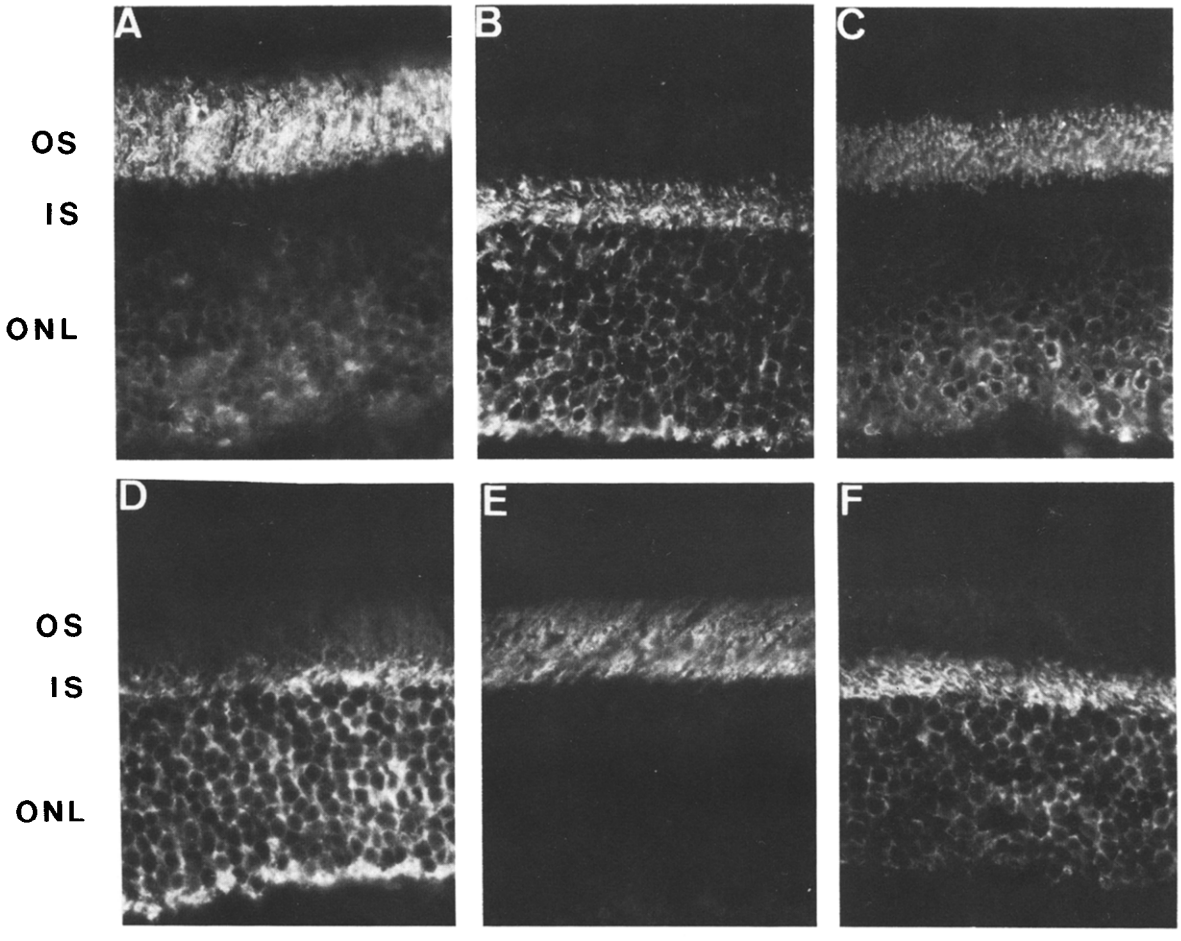

Fig.4. Light-stimulated movement of arrestin and G_α in rat retinas. The tissues shown in A,B,C are stained with anti-arrestin and the tissues in D,E,F are stained with anti-G_α. A and D show the localization of these proteins 3 h after light onset, followed by 3 h of dark (B,E) then 3 h of light (C,F).

light (beginning at the time of normal light onset) followed by 3 h of dark, then 3 h of light. Animals were killed at the end of each 3 h period of light or darkness; the eyes were fixed, sectioned and stained with antibodies to PDE, arrestin and G_α. The results of this experiment indicate that the shifts of both arrestin and G_α can be light-stimulated (fig.4). In addition, shifts in G_α and arrestin were not observed in animals maintained in the dark for 3 h after the time of normal light onset.

In these studies we have shown that two proteins involved in the modulation of PDE activity move between the inner and outer segments in response to ambient lighting conditions. These changes were observed both by gel electrophoresis and immunoblot analysis of purified ROS as well as by immunocytochemistry on tissue sections. This indicates that the distribution of proteins observed on tissue sections cannot be explained solely by a change in antigen-antibody affinity in the dark or in the light. G_α is involved in the stimulation of PDE activity while arrestin acts to quench PDE activity. Movement of G_α and arrestin in response to light would allow for further regulation of receptor sensitivity, thus suggesting a role for protein movement in bleaching adaptation.

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