Monensin Stimulates Glycerolipid Incorporation into Rod Outer Segment Membranes*

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Monensin is an ionophore which disrupts the structure of the Golgi apparatus and inhibits vesicular transport in eukaryotic cells. In this study, we examined the effects of monensin on the incorporation of newly synthesized glycerolipids into retinal rod outer segment (ROS) membranes. Frog retinas were incubated in the presence or absence of monensin (50 nm) with either [1,2,3-3H]glycerol or [9,10-3H]palmitic acid as radiolabeled substrate. Total lipids were extracted from retinas and ROS membranes and resolved into individual phospholipid classes and neutral lipids by thin-layer chromatography. In the presence of monensin, the specific activity of ROS phospholipids was increased about 2-fold with [3H]glycerol and nearly 3-fold with [3H]palmitic acid as substrates relative to controls. In contrast, the specific activity of total retinal lipids, the relative incorporation of label into ROS and retinal phospholipids, and the total lipid phosphorous content of ROS membranes and retinas were not significantly different from control values. These data suggest that the enhanced labeling of ROS phospholipids in the presence of monensin was due to altered intracellular routing of lipids rather than increased glycerolipid synthesis. Under the same conditions, total retinal protein synthesis was about 90% of control, but light microscopic autoradiography indicated that newly synthesized proteins were not transported to the ROS for assembly into disc membranes. Thus, newly synthesized glycerolipids can be delivered to the ROS by a mechanism which is independent of protein transport to that cellular compartment.

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The distinctive molecular composition of each membranous organelle in eukaryotic cells (1, 2) suggests that sorting and targeting mechanisms exist which establish and preserve a differential distribution of membrane constituents in each organelle. However, these mechanisms presently are not well understood. Whereas the biosynthesis, insertion, and intra-cellular translocation of membrane proteins have been examined in considerable detail over the past several years (3-7), relatively little is known about the mechanisms which determine the distribution of lipids among the various membrane compartments of the cell. Two major hypotheses have been put forth to account for the observed stereotypical lipid composition of cellular membranes, one involving differential transport of lipids between membrane compartments via cytoplasmic phospholipid exchange proteins (8-11), and the other involving sequential budding-off and fusion of small membrane vesicles in a process of membrane flow from the endoplasmic reticulum through the Golgi apparatus to the plasma membrane or lysosomes (12-16). The first hypothesis implies that independent mechanisms exist for the intracellular transport of lipids and proteins; whereas the second hypothesis implies that, following their initial synthesis, lipids and proteins remain associated with one another en route to their ultimate destinations within the cell.

Studies of intracellular sorting and translocation of membrane constituents have been greatly aided by the availability of various agents which can selectively perturb specific aspects of these processes. One such agent is monensin (17, 18), a Na⁺ and H⁺ ionophore which has been shown to disrupt the normal morphological integrity of the Golgi apparatus (19-23), to prevent the release of secretory proteins (19, 24-26) and virally encoded membrane proteins (21, 22, 26-28) from cells, and to block the axoplasmic transport of neuronal proteins (29). It has been reported previously (23, 30, 31) that monensin can block the intracellular transport of newly synthesized opsin (the polypeptide moiety of the visual pigment rhodopsin) to the ROS in the rod photoreceptor cells of retinas incubated under conditions where overall protein and glycerolipid syntheses are not appreciably affected. Furthermore, these studies have demonstrated that monensin can produce a gross distention of rod cell Golgi cisternae, as well as a marked accumulation of newly synthesized polypeptides in the Golgi apparatus, indicating that opsin and other newly synthesized proteins are transported to, but not released from, the Golgi apparatus under these conditions.

Although membrane flow may account for the deposition of opsin into ROS membranes (reviewed in Refs. 32, 33, 53, 57, and 58), it is not well established whether lipid constituents of the ROS membranes are exclusively cotransported with opsin or whether some significant fraction of those lipids arrives at the site of ROS membrane assembly by an independent mechanism. Several reports suggest the potential validity of the latter alternative. Autoradiographic (34-41) and biochemical (39-43) studies have indicated that both the

1 The abbreviations used are: ROS, rod outer segment(s); RIS, rod inner segment(s); MeSO₂, dimethyl sulfoxide; PA, phosphatidic acid; PE, phosphatidyethanolamine; PC, phosphatidylcholine; FS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin.
kinetics of labeling of specific rod cell compartments and the relative distribution and turnover of label in those compartments are remarkably different depending on whether protein or lipid precursors are employed. Furthermore, puromycin treatment of retinas has either no significant inhibitory effect or even a slightly stimulatory effect on incorporation of newly synthesized lipids into ROS membranes under conditions where synthesis and intracellular transport of ROS membrane proteins are drastically inhibited (23, 44). In addition, Matheke and Holtzman (23) have employed electron microscopic autoradiography to show that retinas incubated with [3H]glycerol in the presence of monensin can incorporate label into their ROS membranes under conditions where newly synthesized proteins are not transported to the ROS. In this report, we demonstrate that treatment of retinas with monensin in short-term organ culture can cause a significant enhancement in the delivery of newly synthesized polar and neutral glycerolipids to the ROS under conditions where overall retinal glycerolipid synthesis is not stimulated and where protein transport to the ROS is almost completely blocked.

EXPERIMENTAL PROCEDURES

RESULTS

Evaluation of a Usable Concentration of Monensin—Previous studies employing monensin in short-term incubations with isolated retinas (25, 30, 31) have used concentrations in the range 1–10 μM, but little or no information was given concerning the vehicle employed for monensin solubilization or the effects of varying monensin concentrations with regard to retinal protein synthesis. To decide upon a usable monensin concentration, the following criteria were established: (a) no significant cytotoxicity, (b) specific and characteristic morphological effects on the Golgi apparatus in photoreceptor cells, and (c) inhibition of opsin assembly into ROS membranes. We evaluated the first criterion by monitoring the effect of increasing concentrations of monensin on protein synthesis, as measured by the incorporation of [3H]leucine into retina acid-precipitable material. In our studies, a stock solution of monensin dissolved in Me2SO was serially diluted with Me2SO prior to addition to the incubation medium. As shown in Fig. 1, monensin significantly inhibited protein synthesis when present at a concentration greater than 50 nM under the given short-term culture conditions. At a monensin concentration of 50 nM, the incorporation of [3H]leucine was about 90% of control. Further indication that this concentration of monensin was not cytotoxic to retinal cells was demonstrated by light microscopy of control (Fig. 2A) and monensin-treated retinas (Fig. 2B). At the light microscopic level, monensin-treated retinas exhibited a morphological appearance comparable to that of controls.

Monensin has been shown to cause a characteristic swelling of the Golgi apparatus in various cell types (17, 19–23, 30), including retinal photoreceptor cells (23, 30). The normal orderly stacking of Golgi membranes is replaced by a gross dilation of the cisternae, forming vacuoles of various sizes. Such characteristic morphological changes were apparent in electron micrographs of retinas incubated with 50 nM monensin.

2 "Experimental Procedures" are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M 4219, cite the authors, and include a check or money order for $1.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

Fig. 1. Effect of monensin on incorporation of [3H]leucine into retina acid-precipitable material. Frog retinas were preincubated (without isotopes) for 1 h at 24°C in a bicarbonate-buffered modified Ringer’s medium containing monensin (concentration range 5 nM to 5 μM) and 0.25% (v/v) Me2SO under an atmosphere of 95% O2, 5% CO2. Control medium (no monensin) contained 0.25% Me2SO. Retinas were transferred to fresh medium containing [3H]leucine (9 μCi/ml), and the incubations were continued an additional 4 h (maintaining the same monensin and Me2SO concentrations as used in the preincubation). Retinas were then individually sonicated in ice-chilled 10% chloroform and the acid-precipitable material was collected by centrifugation and solubilized in 1 ml of 1 N NaOH (18 h at 65°C); aliquots were then taken for measurement of radioactivity and protein content. Data represent the mean values (± S.D.) of the specific activity (dpm/μg of protein) of acid-precipitable material, expressed as percent of the control value. The number of independent samples per treatment is given above each bar.

Monensin Conc.

| Concentration (μM) | Specific Activity (dpm/μg protein) |
|-------------------|----------------------------------|
| 0                 | 100%                             |
| 0.1               | 80%                              |
| 1                 | 60%                              |
| 5                 | 40%                              |
| 50                | 20%                              |

The myoid region of rod inner segments was virtually filled with large unilamellar vesicles not observed in controls (Fig. 2C). In addition, the mitochondria of monensin-treated retinas appeared serpentine and condensed relative to controls, as previously observed (23, 30). Some rods in both the monensin-treated and control retinas displayed a variable separation between the inner and outer segments, as well as some swelling of the nuclear envelope; these features are capricious artifacts commonly observed in retinas which have been maintained in vitro for several hours.

The third criterion was evaluated by incubating retinas with [3H]leucine in the presence or absence of monensin. The presence of a concentrated "band" of silver grains at the base of the ROS in light microscopic autoradiograms is characteristic of the assembly of newly synthesized proteins into ROS disc membranes (34, 35), whereas the absence of such bands indicates a lack of new membrane assembly. As shown in the autoradiograms in Fig. 3, silver grains were concentrated at the base of the ROS in control retinas (Fig. 3A); heavy but more diffuse labeling of the RIS as well as other cellular layers was also observed, indicative of vigorous protein synthesis in all retinal cell types. In contrast, retinas incubated in the presence of 50 nM monensin did not exhibit distinct bands of silver grains at the base of the ROS (Fig. 3B); instead, silver grains appeared to be concentrated over the myoid region of the RIS. These observations are consistent with those previously reported (23, 30, 31) for retinas incubated in a monensin concentration 100-fold greater than that employed in this study. In one of the former studies (23), quantitative electron microscopic autoradiographic analysis revealed that the monensin-induced congestion of newly synthesized proteins in the myoid region was due to a lack of release of those proteins from the Golgi apparatus, thus explaining the inhibition of intracellular transport of these constituents to the ROS.
Monensin and ROS Membrane Glycerolipid Incorporation

FIG. 2. Light and electron micrographs of retinas incubated for 5 h in the absence (A and C) or presence (B and D) of 50 nM monensin. No morphological differences are apparent at the light microscopic level between control (A) and monensin-treated (B) retinas. However, at the electron microscopic level, note the presence of numerous vacuoles (distended Golgi complexes and smooth endoplasmic reticulum, denoted by open arrows) in the myoid region (M) and the condensed, serpentine appearance of mitochondria in the ellipsoid region (E) of the RIS in the monensin-treated tissue (D). See “Experimental Procedures” for details. Scale bars = 30 μm (A and E) and 5 μm (C and D). ONL, outer nuclear layer; INL, inner nuclear layer.

Having satisfied the three criteria outlined above, we used 50 nM monensin throughout the remainder of this study to examine whether or not this drug has any effects on retinal lipid synthesis or the incorporation of de novo synthesized lipids into ROS membranes under conditions where protein assembly into ROS membranes does not occur.

Table I

| Sample | Lipid phosphorus | Specific activity |
|--------|------------------|-------------------|
|        | μg               | dpm/μg lipid P (×10^4) |
| Retina |                  |                   |
| Monensin | 4.80 ± 0.69   | 2.81 ± 0.19       |
| +Monensin | 3.88 ± 0.40   | 3.55 ± 0.73       |
| % of control | 81% (NS)*   | 126% (NS)         |
| ROS     |                  |                   |
| Monensin | 4.11 ± 0.55   | 2.22 ± 0.50       |
| +Monensin | 4.06 ± 0.88   | 4.87 ± 1.45       |
| % of control | 99% (NS)      | 219% (p < 0.01)   |

*NS, not significant (Student’s t test).

Having satisfied the three criteria outlined above, we used 50 nM monensin throughout the remainder of this study to examine whether or not this drug has any effects on retinal lipid synthesis or the incorporation of de novo synthesized lipids into ROS membranes under conditions where protein assembly into ROS membranes does not occur.

FIG. 3. Light microscopic autoradiograms of retinas incubated with [3H]leucine in the absence (A) or presence (B) of 50 nM monensin. Note the band of silver grains (solid arrows, A) at the base of the ROS in control retina and the absence of such a band in the monensin-treated tissue. Also note the concentration of silver grains over the myoid compartment of the RIS (open arrows, panel B) in monensin-treated tissue compared with the intense but relatively even distribution of silver grains over the RIS in control tissue. See “Experimental Procedures” for details. Scale bar = 15 μm for A and B.

Table I

Effect of monensin on [3H]glycerol incorporation into lipids of frog retinas and ROS membranes

Retinas (20 per flask) were incubated as described under “Experimental Procedures” in medium containing [1,2,3-3H]glycerol (0.2 mCi/ml, 5.2 μCi/μM) and 0.25% MgSO_4 with or without monensin (50 nM). After brief rinsing in ice-chilled fresh medium (3 × 10 ml, containing 1% glycerol), four retinas from each flask were processed as follows. One-half of each was processed for microscopy, whereas lipids were extracted from the other half with chloroform/methanol/water (10:20:8). The remaining 16 retinas from each flask were used to obtain four individual ROS preparations by discontinuous sucrose gradient centrifugation, and the ROS membranes were extracted to obtain the lipids. Aliquots of each lipid extract were assayed in triplicate for determination of lipid phosphorous content and radioactivity. Results are expressed as the mean ± S.D. (n = 4).

| Sample | Lipid phosphorus | Specific activity |
|--------|------------------|-------------------|
|        | μg               | dpm/μg lipid P (×10^4) |
| Retina |                  |                   |
| Monensin | 4.80 ± 0.69   | 2.81 ± 0.19       |
| +Monensin | 3.88 ± 0.40   | 3.55 ± 0.73       |
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| Monensin | 4.11 ± 0.55   | 2.22 ± 0.50       |
| +Monensin | 4.06 ± 0.88   | 4.87 ± 1.45       |
| % of control | 99% (NS)      | 219% (p < 0.01)   |

*NS, not significant (Student’s t test).
relative to controls, based on a comparison of specific activity values. Therefore, overall de novo biosynthesis of retinal glycerolipids was neither stimulated nor inhibited by the drug. In contrast, monensin enhanced the incorporation of \([\text{H}]\)glycerol into ROS membrane lipids more than 2-fold relative to controls (219%, \(p < 0.01, n = 4\)). The mass of phospholipid analyzed from the corresponding samples of each group was virtually identical; hence, this marked difference in specific activity values of the ROS lipid extracts was due solely to an elevation in the incorporated radioactivity.

Aliquots of the lipid extracts obtained from ROS membranes were analyzed by two-dimensional TLC. When the results were compared on the basis of the relative distribution of label among the various glycerolipid classes, with the incorporation of \([\text{H}]\)glycerol into each class expressed as a percent of the total radioactivity in the lipid extract (Table II), there were no major differences observed in the presence or absence of monensin. That is to say, no particular phospholipid class exhibited a selective enhancement of labeling in the presence of monensin. Furthermore, the relative incorporation of label into total phospholipids versus total neutral lipids did not differ significantly as a function of the presence of the drug. In both the control and monensin-treated samples, at least two-thirds of the total radioactivity incorporated into ROS membrane lipids was found in the neutral lipid fraction. A similarly preferential labeling of the neutral glycerides of both whole retinas and ROS membranes has been reported previously by Bazan et al. (49–51) for both bovine and toad retinas incubated in short-term organ culture with radiolabeled glycerol. When the values for the radioactivity incorporated into each ROS phospholipid class were normalized to the total lipid phosphorous content of the lipid extract analyzed (Fig. 4), PI, PC, PE, and the neutral lipids each exhibited nearly a 2-fold increase (\(p < 0.05\)) in incorporated radioactivity in the presence of monensin relative to controls. These lipid classes represent about 85–90% of the total lipid mass of ROS membranes (62, 53). Although PA and PS both showed a trend toward enhanced labeling in the presence of monensin, the increase was not statistically significant.

\([\text{H}]\)Palmitate Incorporation into Retinal and ROS Membrane Phospholipids—To confirm the above findings with an independent, general glycerolipid precursor, we examined the incorporation of \([\text{H}]\)palmitic acid into the phospholipids of retinas and ROS membranes in an analogous manner. In this experiment, the neutral lipids (including free \([\text{H}]\)palmitic acid) were separated from the phospholipids by one-dimensional TLC, and the radioactivity and lipid phosphorous content of the phospholipids (which remained at the origin) were then measured. The results are shown in Table III. Based upon both specific activity and relative percent incorporation values, there was no significant effect of monensin...
on the incorporation of \(^{[3]H}\)palmitic acid into total retinal phospholipids relative to controls. This indicated that monensin did not stimulate phospholipid synthesis in the retina, consistent with the results of the previous experiment which utilized \(^{[3]H}\)glycerol as the radiolabeled lipid precursor. However, the specific activity of ROS membrane phospholipids from retinas incubated with monensin was nearly 3-fold higher (297%, p < 0.005, n = 4) than that of controls. In contrast, total ROS phospholipid mass and the relative percent incorporation values were not significantly different from control values.

**DISCUSSION**

Using both light microscopic autoradiography and biochemical analysis, we have demonstrated that retinas incubated with \(^{[3]H}\)leucine in the presence of 50 nm monensin failed to incorporate newly synthesized proteins into ROS membranes, even though retinal protein synthesis was not significantly depressed relative to controls. Under these same conditions, the results of experiments employing \(^{[3]H}\)glycerol and \(^{[3]H}\)palmitic acid as glycerolipid precursors indicate that monensin caused at least a 2-fold increase in the incorporation of newly synthesized glycerolipids into ROS membranes, whereas overall retinal lipid synthesis was not appreciably stimulated. The enhanced lipid incorporation was relatively uniform among all major glycerolipid classes, and there was no compensatory shift in the incorporation of radiolabeled glycerol into neutral lipids versus phospholipids which could account for this enhanced labeling of the total ROS membrane lipids. We propose that a redistribution of cellular lipid pools occurred in the presence of monensin under the given *in vitro* conditions such that routing of glycerolipids to the ROS was enhanced at least 2-fold relative to controls. Thus, the rod cell appears to have a remarkable degree of plasticity with regard to the sorting and targeting of its membrane constituents. This implies that one or more alternative cellular repositories of these lipids were compensatorily depleted. Consistent with this hypothesis, Mathke and Holtzman (23), using quantitative electron microscopic autoradiography, reported that labeling of the synaptic endings of rods with \(^{[3]H}\)glycerol in the presence of monensin was about 30% lower than in controls. Our results both confirm and extend some previous observations concerning the synthesis and intracellular transport of lipids and proteins destined for ROS membrane assembly.

The retinal rod photoreceptor is a highly differentiated and polarized neuronal cell and has been examined extensively by biochemical, morphological, and electrophysiological methods (reviewed in Refs. 33, 52–58, and 61). The ROS consists of an orderly stack of several hundred flattened membranous sacs (the so-called "disc membranes") surrounded by the plasma membrane of the cell. ROS membranes consist almost entirely of lipids and proteins, in approximately equal amounts by weight. Rhodopsin, the rod visual pigment, accounts for greater than 95% of the total ROS integral membrane protein; whereas glycoporphospholipids represent about 90% of the total ROS lipid. The RIS contains the organelles and most of the enzymes responsible for cellular metabolism and is joined to the ROS by a narrow cilium. It is through this cilium that exchange of cellular constituents between the RIS and ROS occurs. Opsin is initially synthesized and glycosylated on the rough endoplasmic reticulum and is then transported to the Golgi apparatus where its two N-linked oligosaccharide chains undergo their final maturation. Opsin is transported from the Golgi apparatus through the RIS to the periciliary RIS plasma membrane via a mechanism thought to involve specialized membrane vesicles (33, 59, 60). These vesicles apparently fuse with a discrete domain of the RIS plasma membrane near the connecting cilium (33, 61, 62); subsequently, opsin diffuses to the base of the ROS where the ciliary plasma membrane expands to form nascent disc membranes (33, 63). The retinaldehyde prosthetic group becomes coupled to the protein to form rhodopsin only after opsin has been delivered to the base of the ROS (see Ref. 58). Thus, opsin is associated with lipids throughout its lifetime in the rod cell (64), and its transport through the RIS to the ROS is consistent with a mechanism involving membrane flow.

Whereas the details of rhodopsin synthesis, intracellular transport, and assembly into ROS membranes are relatively well understood, these aspects of photoreceptor lipid metabolism have not yet been defined with comparable clarity. From the above discussion, one might assume that fusion of opsin-containing vesicles with the plasma membrane and subsequent membrane flow might also account for the delivery of lipids to the ROS. However, it is not clear that such vesicular transport and membrane fusion adequately explain the lipid content or composition of the ROS membranes. The results of quantitative freeze-fracture electron microscopy studies (69, 65) have demonstrated that the density of intramembrane particles in the ROS disc membranes is about twice that of the ROS vesicles which apparently transport opsin to the plasma membrane (33, 60), whereas the intramembrane particle size distribution in both the vesicles and disc membranes is comparable. The intramembrane particles in these membranes are thought to represent primarily opsin, whereas the intervening areas represent lipid domains. Thus, the constituents of the vesicles which transport opsin to the cell surface are not simply inserted *en bloc* into nascent disc membranes with preservation of their original composition and architecture. Furthermore, as mentioned previously (see the Introduction), the results of several studies have indicated that the dynamics of lipids in photoreceptor cells are quite distinct from those of proteins, especially with regard to the kinetics of intracellular transport to and the relative turnover rates of these constituents in the ROS membranes (34–43). In particular, studies employing inhibitors of protein synthesis and/or transport (23, 30, 31, 44) have yielded results which demonstrate that glycerolipids can be synthesized and transported to the ROS under conditions where protein synthesis and/or transport are almost completely abolished. As previously suggested by Holtzman and co-workers (25, 32, 41, 66), the photoreceptor cell has elaborated alternate mechanisms of glycerolipid transport which do not involve the endoplasmic reticulum → Golgi apparatus → plasma membrane pathway utilized for the transport of integral membrane proteins (e.g., opsin) in the cell.

There are two likely mechanisms which would account for the independent transport of glycerolipids: (a) membrane flow involving an alternative route (i.e., by-passing the Golgi apparatus), and (b) phospholipid exchange proteins. With regard to the first alternative, reports by Mercurio and Holtzman (41, 66) have provided evidence which suggests that the endoplasmic reticulum network of the rod cell is considerably more complex than previously appreciated. In particular, there exists a specialized type of smooth endoplasmic reticulum (the "subellipsoid smooth endoplasmic reticulum") which has continuities with the rough endoplasmic reticulum in the myoid region, but is not continuous with the Golgi apparatus. Thus, there exists a potential structural basis for alternative membrane flow within the rod cell. These authors have demonstrated that the majority of glycerolipid synthesis in the
rods takes place in the rough endoplasmic reticulum and nuclear envelope and that the Golgi apparatus shows only minimal labeling with \(^{3}H\)glycerol even over extended incubation times (41). In contrast, the rough endoplasmic reticulum and Golgi apparatus become heavily labeled in the presence of \(^{3}H\)leucine. Thus, newly synthesized lipids and proteins originate in the same organelle of the rod cell, but the proportion of each molecular class and its kinetics of transport through the various intracellular compartments differ greatly.

With regard to the second alternative, a protein fraction isolated from bovine retina has been shown to have the capacity to catalyze the exchange of phospholipids between radiolabeled PC liposomes and ROS membranes (67). This retinal protein fraction exhibits a specificity for ROS membranes as acceptors relative to other cellular membranes (e.g. mitochondria), but otherwise has not been well characterized. Furthermore, the participation of exchange proteins in the “molecular replacement” of disc membrane lipids has been postulated in biochemical and autoradiographic studies of the turnover of these lipids (reviewed in Refs. 53 and 57). Although the existing literature and our results suggest the existence of multiple pathways for lipid transport in the rod cell, none of the studies performed to date has clearly indicated a preferential utilization of one pathway versus another for the transport of lipids from the RIS to the ROS. Multiple transport pathways would necessitate some regulatory mechanism for coordinating the supply of membrane constituents to the site of ROS membrane assembly. However, such a regulatory mechanism has yet to be elucidated.

One cannot infer from our results that ROS membranes formed in the presence of monensin are necessarily protein-deficient or lipid-enriched relative to the ROS membranes of control retinas. Autoradiographic studies have shown that radiolabeled lipid precursors, unlike protein precursors, are not incorporated as bands at the base of the ROS (23, 36-41). Rather, the entire ROS becomes diffusely labeled. This is because the ROS membrane lipids, unlike the proteins, are free to exchange throughout the entire ROS membrane system (by a mechanism which has yet to be fully revealed) rather than remaining confined to the disc membranes into which they were originally incorporated at the base of the ROS (reviewed in Refs. 53 and 57). Thus, it is likely that any new discs formed in the presence of monensin would contain a mixture of new and “old” (i.e. previously synthesized) lipids as well as old proteins. Assuming that the newly delivered lipids were distributed uniformly throughout the ROS membranes, the mass of that lipid would be almost negligible in comparison with the previously existing lipid mass of the ROS. Hence, even in the presence of monensin, no significant lipid enrichment of ROS membranes would have occurred during the 5-h incubation employed in this study. If, however, some significant fraction of the newly delivered lipids were somehow sequestered for elaboration of new discs, osin and other requisite membrane proteins could be recruited from the ROS plasma membrane by lateral diffusion (68, 69) to support normal disc morphology. Calculations indicate that the ROS plasma membrane contains sufficient osin to support disc membrane assembly for several hours. For example, in the frog retina, the ROS plasma membrane represents about 1% of the total ROS membrane area (i.e. equivalent to about 17-20 discs), and the average rate of new membrane assembly is about 1-1.5 discs/rod/h (see Refs. 54, 56, and 57). Therefore, over a 5-h period, each rod would normally assemble between 5 and 7.5 new discs, which only corresponds to about 25-42% of the membrane area of the ROS plasma membrane.

Our results and those of Holtzman's laboratory (23, 41) have indicated that some significant percentage of glycerolipids synthesized by the rod cell by-pass the Golgi apparatus. Using a variety of metabolic inhibitors including monensin, Sleigh and Pagano (70) have reported analogous studies which demonstrate the independent transport of PE and proteins to the plasma membrane in cultured Chinese hamster fibroblasts. Interestingly, other studies with cultured fibroblasts from Pagano’s laboratory (71-73), employing fluorescent derivatives of sphingolipids, have indicated that sphingolipids are routed through the Golgi apparatus prior to their subsequent distribution throughout the cell and that the appearance of labeled lipid at the cell surface can be inhibited by 10 µM monensin. Therefore, it appears that glycerolipids and sphingolipids may be routed through the cell by somewhat different mechanisms. It remains to be seen whether rod cells also exhibit this differential routing of glycerolipids and sphingolipids and to elucidate the mechanism by which such differential transport is achieved.

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Supplementary Material to
RODENES STIMULATES GLYCEROPLIPID INCORPORATION INTO ROS OUTER SEGMENT MEMBRANES
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Procedures (see below)
Monensin and ROS Membrane Glycerolipid Incorporation

Analysis of Lipid Extracts

Aliquots of each lipid extract were taken for measurement of incorporated radioactivity and for determination of lipid phosphorus content (4R). After collecting the amount of phospholipid in each sample, aliquots of each sample were resolved into their constituent lipid classes by TLC. Neutral lipid classes were separated from phospholipid by one-dimensional TLC on silica gel 60 plates using the solvent system benzene/diethyl ether/glacial acetic acid (70:30:1, by vol.). A mixture of authentic neutral lipid standards containing approximately 20 μg each of monolein, diglyceride, oleylethanolamine, cholesteryl oleate, and cholesterol was chromatographed with each sample as well as in an adjacent lane. After visualization of the lipid components by exposure to iodine vapor and comparing thelane to that of the neutral lipid standard mixture, the individual neutral lipid classes were scraped into glass vials containing scintillation cocktail and analyzed for radioactivity, while the phospholipids (which remained at the origin) were scraped from the plate into scintillation vials and counted for lipid phosphorus content and radioactivity. Individual phospholipid classes were resolved by two-dimensional TLC on precoated silica gel 60F254 plates, using the solvent system chloroform/methanol/28% ammonium hydroxide (60:30:16, by vol.) in the first dimension and chloroform/methanol/0.1% acetic acid/0.85% water/acetic acid (95:5:2:20:3:1:6, by vol.) in the second dimension. The plates were dried under nitrogen for 15 minutes between each development. To facilitate the visualization and localization of lipid components, each sample was chromatographed with an internal standard mixture of phospholipids containing 100 μg of each of the neutral lipid standard mixture. Lipid components were visualized by exposure to iodine vapor and then scraped from the plates into scintillation vials for measurement of radioactivity. Radioactivity values were normalized to the amount of total radioactivity recovered from the TLC plate.