Light- and Cytidine-dependent Phosphatidylinositol Synthesis in Photoreceptor Cells of the Rat

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ABSTRACT Incorporation of [^{3}H]inositol into phosphatidylinositol (PI) in isolated rat retinas is enhanced by light and by the addition of cytidine to the incubation media. In retinas preincubated with [^{3}H]inositol in dark, [^{3}H]inositol was chased into PI in light by addition of unlabeled cytidine and was chased out of PI in light by addition of unlabeled cytidine plus inositol. Autoradiograms of retinas show a heavy density of silver grains over photoreceptor cell inner segments (with chase-in) and a loss of labeling (with chase-out). Exogenous cytidine and inositol were shown to enhance not only the turnover of PI within photoreceptor cells but the synthesis of PI as well; in media supplemented with these precursors, ~50% of [^{14}C]glycerol and 25% of [^{32}P$_i$] incorporated into lipid in light were associated with PI. These results suggest that availability of both cytidine and inositol may play a role in the light-dependent changes in PI metabolism within photoreceptor cells.

Previous studies have shown that light enhances both synthesis (6, 11) and turnover (10) of phosphatidylinositol (PI)[1] in isolated rat retinas. Although pathways of PI metabolism have not been defined in rat retinas, the evidence indicates (see preceding paper, reference 9) that a cytidine-containing phospholipid intermediate cytidine diphosphate–diacylglyceride (CDG) is a precursor for PI and that conversion of CDG to PI is enhanced in light particularly within the inner segments of photoreceptor cells. In retinas incubated with tracer amounts of [^{3}H]cytidine, [^{3}H]cytidine was incorporated into CDG within photoreceptor inner segments, and into RNA within photoreceptor nuclei (9). ^{3}H-labeled CDG was shown to accumulate, within photoreceptor cell inner segments, only in dark or when RNA synthesis was inhibited by actinomycin D. These observations suggested that endogenous cytidine or tracer amounts of [^{3}H]cytidine may be limiting for CDG and PI synthesis in light as cytidine was used preferentially for RNA synthesis.

In view of previous reports that showed both cytidine-dependent and cytidine-independent mechanisms for PI synthesis in hepatocytes (3, 8) and cultured glial cells (5), the present studies were done to determine the role of cytidine-dependent and cytidine-independent pathways in the light-enhanced synthesis and turnover of PI in photoreceptor cells of the rat. The effects of cytidine on PI synthesis and turnover, respectively, were evaluated in retinas incubated with [^{14}C]-glycerol and ^{32}P$_i$ in dark or light, and in chase incubations of retinas preincubated with [^{3}H]inositol. The site of light- and cytidine-dependent synthesis and turnover of PI was determined by microdissecting retinas into photoreceptor cell and inner retina layers and by autoradiography.

[1] *Abbreviations used in this paper:* CDG, cytidine diphosphate-diacylglyceride; L/D ratio, light vs. dark ratio; PI, phosphatidylinositol.

MATERIALS AND METHODS

Materials: Materials were the same as in the preceding paper (9). In addition, [^{3}H]inositol, [^{14}C]glycerol, and [^{32}P$_i$]orthophosphoric acid were purchased from New England Nuclear (Boston, MA). Myoinositol, cytidine, and other reagent grade chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Retina Incubation and Phospholipid Extraction: Retinas were removed in dim red light from dark-adapted 26- to 32-day-old Long-Evans rats and were incubated with [^{3}H]inositol, [^{3}H]cytidine, and [^{14}C]inositol, or [^{14}C]glycerol (20 μCi/ml; 144 Ci/mmol) and ^{32}P$_i$ (15.6 disintegrations per minute [dpm]/10^{-18} mol) in dark or light under the conditions detailed in the preceding paper (9). Incorporation of [^{3}H]inositol into PI was evaluated in retinas incubated for 30 min in dark or light in the presence of various concentrations of [^{3}H]inositol (2×10^{-6} M–10^{-2} M) with or without added cytidine (10^{-4} M or 1.5×10^{-3} M). The effects of added cytidine (1.5×10^{-3} M), inositol (10^{-2} M) or cytidine plus inositol on the incorporation of [^{14}C]-inositol and [^{3}H]cytidine into PI and CDG, respectively, were measured simultaneously in double label incubations. Incorporation of [^{14}C]glycerol and ^{32}P$_i$ into lipids was determined in retinas incubated in dark or light in the standard medium and in the same medium supplemented with unlabeled 1.5×10^{-3} M cytidine plus 10^{-2} M inositol (enriched medium).

After 30 min of incubation in dark or light, retinas were rapidly washed free

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of radioactive medium (in two 150-ml volumes of ice-cold 0.9% saline containing cytidine [1%], inositol [1%], and formaldehyde [2%]), were frozen individually in liquid nitrogen, freeze-dried, weighed, and stored until analysis (5–8 d) at −80°C. In some experiments, retinas were microdissected into photoreceptor cell and inner retina layers as previously described (10, 11). Before extraction of lipids, retinal samples were homogenized in 0.5 ml of 2 M KCl and the radioactivity of the homogenate was determined (duplicate 10-μl aliquots) as a measure of precursor uptake into the retina during incubation. Lipids were extracted in acidified chloroform: methanol solvent and the radioactivity of the final washed extract was quantitated (duplicate 25-μl aliquots were counted) as a measure of precursor incorporation into total lipid. The various lipid classes were separated by two-dimensional thin-layer chromatography on silica gel 60 coated plastic sheets. Radioactive lipids were localized on thin-layer chromatography sheets by autoradiography (Kodak AR, x-ray film), and the spots were marked on each sheet, cut out, solubilized, and counted. Incorporation of [^{3}H]- or [^{14}C]inositol into PI was determined from the radioactivity of the extract and from the fraction of the total radioactivity associated with the PI spot on each given thin-layer chromatography sheet. In a similar manner, ^{14}C- or $^{32}P_i$-radioactivity associated with a given lipid spot was taken as a measure of incorporation of [^{14}C]glycerol or $^{32}P_i$ into that particular lipid. Incorporation of [^{3}H]cytidine into CDG was directly quantitated from the radioactivity of extracts of retinas incubated with [^{3}H]cytidine. Data are expressed as disintegrations per minute or picomoles of precursor incorporated per whole retina or per milligram retina.

In some experiments, the retinas were microdissected, and the total phospholipid phosphorus content was determined in phospholipid extracts of photoreceptor cell and inner retina layers, and results are expressed as disintegrations per minute incorporated per nanomoles phospholipid phosphorus (10, 11).

Chase Incubations and Autoradiography: Pilot studies on autoradiographic localization of incorporation of [^{3}H]inositol into PI in dark or light in isolated rat retinas yielded variable and inconsistent results. Therefore, studies were undertaken to standardize the incubation conditions and to localize, through chase incubations, the sites of light-enhanced turnover of PI in isolated rat retinas. The cycle of PI turnover (see Fig. 7 of reference 9) suggested that, in retinas preincubated with [^{3}H]inositol in dark, [^{3}H]inositol could be chased into PI in light and in the presence of cytidine, while [^{3}H]-inositol could be chased out of PI in light and in the presence of cytidine plus inositol. In the first instance, light and cytidine would enhance the incorporation of the accumulated [^{3}H]inositol into PI, while, in the second instance, light-enhanced turnover of PI (involving hydrolysis of PI and increased incorporation of unlabeled inositol into PI) would be reflected in loss of radioactivity from prelabeled [^{3}H]PI.

Retinas (four to six retinas per 0.6–1 ml of medium) were preincubated with [^{3}H]inositol (1.5 mCi/ml; 0.5×10^{-3} M) for 30 min in dark, and then the incubation medium was diluted 40-fold with media containing unlabeled cytidine, inositol, or cytidine plus inositol, and chase incubations were conducted for 30 min in dark or light. Similar chase incubations were also done with retinas that were preincubated in dark with [^{3}H]cytidine (250 μCi/ml; 10^{-5} M) to obtain a measure of CDG turnover in isolated rat retinas. Chase incubations were terminated by addition of fixative. One to two retinas were prepared for autoradiography, and three to four retinas were frozen for microdissection and biochemical analysis. The amounts of [^{3}H]inositol or [^{3}H]-cytidine incorporated into PI or CDG were quantitated as described above. All experiments were repeated three times. Autoradiography was done according to the method of Gould and Dawson (7) as described in the preceding paper (9). Representative autoradiograms are presented after 6 d of exposure to photographic emulsion.

RESULTS

Table I shows that incorporation of [^{3}H]inositol into PI during 30 min of incubation in dark or light increased as the concentration of inositol was increased in the incubation medium. The rate of incorporation of [^{3}H]inositol plotted versus inositol concentration in the medium appeared to be saturable with maximal velocity (V_{max}) values that were similar in dark and light (630 vs. 880 pmol per retina per 30 min) and Michaelis constant (K_m) values that were 0.7×10^{-3} M in light and 2×10^{-3} M in dark. At low concentrations of inositol ($<0.5 \times 10^{-3}$ M), incorporation of [^{3}H]inositol into PI was enhanced in light, whereas, at higher concentrations ($\geq 10^{-3}$ M), light-dependent increases in [^{3}H]inositol incorporation could not be detected unless the medium was supplemented with cytidine.

TABLE I

Incorporation of [^{3}H]Inositol into PI in Rat Retinas Incubated in Dark or Light as a Function of Medium Inositol and Cytidine Concentration

Additions to the incubation		[^{3}H]Inositol incorporated into PI		L/D
Inositol	Cytidine	Dark	Light	Ratio
		pmol/retina/30 min		
2×10^{-6} M	—	0.76 ± 0.1	1.58 ± 0.3	2.08
4×10^{-5} M*	—	14 ± 2.6	29 ± 5.6	2.07
10^{-4} M	—	40 ± 7.0	74 ± 14	1.85
0.5×10^{-3} M	—	191 ± 32	306 ± 46	1.60
10^{-3} M	—	292 ± 58	321 ± 51	1.10
2×10^{-3} M	—	554 ± 87	490 ± 91	0.88
5×10^{-3} M	—	646 ± 104	680 ± 120	1.05
10^{-2} M	—	764 ± 122	839 ± 128	1.10
2×10^{-6} M	10^{-4} M	1.23 ± 0.2	2.76 ± 0.4	2.24
2×10^{-6} M	1.5×10^{-3} M	1.72 ± 0.3	2.47 ± 0.4	1.43
0.5×10^{-3} M	10^{-4} M	239 ± 41	616 ± 108	2.58
10^{-2} M	1.5×10^{-3} M	908 ± 161	1,698 ± 307	1.87

Retinas were incubated for 30 min in dark or light with [^{3}H]inositol (25 μCi/ml, 12.5 Ci/mmol). The rates of incorporation were calculated from the initial specific radioactivities (dpm/pmol) of the medium [^{3}H]inositol. The values represent the mean ± SD for six to eight analyses.

* Inositol concentration in medium comparable to that of medium containing ^{14}C-labeled inositol.

Addition of cytidine to media containing [^{3}H]inositol (2×10^{-6} M, 0.5×10^{-3} M, or 10^{-2} M) resulted in rates of [^{3}H]-inositol incorporation in dark that were significantly higher than those observed in the absence of cytidine. The light vs. dark (L/D) ratios for [^{3}H]inositol incorporation into PI were the highest in media containing low levels of both inositol and cytidine (2×10^{-6} M, or 0.5×10^{-3} M inositol plus 10^{-4} M cytidine). The L/D ratios were reduced when cytidine was limiting for PI synthesis (i.e., in media without exogenous cytidine but containing 0.5×10^{-3} M or higher levels of inositol) or when inositol was limiting for PI synthesis (i.e., in media without exogenous inositol, but containing an elevated level of cytidine, 1.5×10^{-3} M).

Total tissue radioactivity as a measure of [^{3}H]inositol uptake was similar in retinas in dark and light. In media containing 10^{-3} M inositol or less, tissue-to-medium ratios were close to 1 within 30 min of incubation. In media containing higher concentrations ($>10^{-3}$ M), tissue-to-medium ratios were below one (0.75–0.88).

Incorporation of [^{3}H]cytidine and [^{14}C]inositol into CDG and PI, respectively, measured in double-label incubations, is shown to be affected by the addition of either unlabeled cytidine (1.5×10^{-3} M), inositol (10^{-2} M), or inositol plus cytidine to the incubation (Fig. 1). In media supplemented with cytidine only, ^{3}H-labeled CDG was increased 330-fold in dark and 470-fold in light, while the incorporation of [^{14}C]inositol into PI was enhanced in dark but not in light compared to retinas incubated in the standard medium in dark and light, respectively. Under these conditions, the levels of endogenous inositol appeared to be limiting for light-enhanced incorporation of inositol into PI, and the L/D ratio for [^{14}C]PI was reduced (1.4 in the presence of added cytidine compared with 2.1 in the standard incubation). In the presence of added inositol (10^{-2} M), incorporation of [^{14}C]inositol into PI was increased 55-fold in dark and 32-fold in light

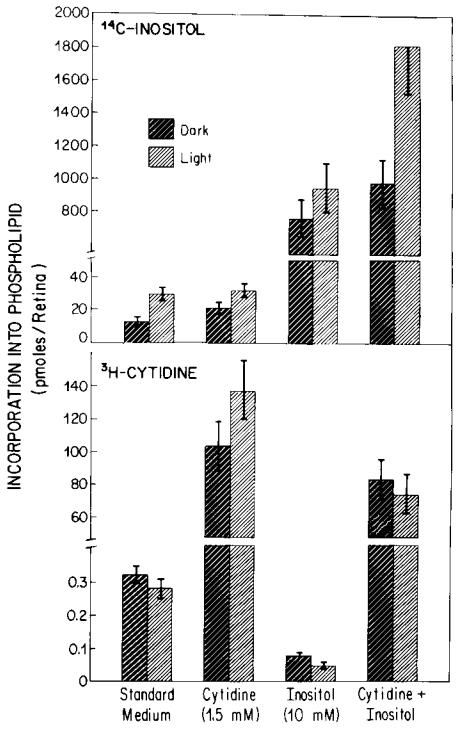

FIGURE 1 Incorporation of [^{3}H]cytidine (25 μCi/ml; 26 Ci/mmol) into CDG, measured simultaneously with incorporation of [^{14}C]-inositol (10 μCi/ml; 250 mCi/mmol) into PI in retinas incubated for 30 min in dark or light. Incubations were carried out in the standard medium or in medium supplemented with cytidine (1.5×10^{-3} M), inositol (10^{-2} M), or cytidine plus inositol. Data for incorporation of these precursors was calculated from the initial specific activity (dpm/pmoles) of [^{14}C]inositol or [^{3}H]cytidine in each medium and from the radioactivity of [^{3}H]CDG and [^{14}C]PI in extracts of retinas. The bars represent the mean and the vertical lines within the bars ± SD for four to six determinations.

compared with standard incubations, concurrently with 60% reductions in the levels of [^{3}H]CDG. Under these conditions cytidine appeared to be limiting for light-enhanced incorporation of [^{14}C]inositol into PI, and the L/D ratio for [^{14}C]PI was reduced to 1.2.

Incorporation of [^{14}C]inositol into PI was enhanced in light in media supplemented with both unlabeled inositol (10^{-2} M) and cytidine (1.5×10^{-3} M), and the L/D ratio was close to 2. Light-enhanced incorporation of [^{3}H]cytidine into CDG was detected only under conditions when cytidine was present in excess (i.e., in media supplemented with cytidine only), and the L/D ratio for [^{3}H]CDG was close to 1.4. The accumulation of [^{14}C]inositol and [^{3}H]cytidine did not appear to be a factor in the observed differences in their incorporation since total tissue radioactivities were similar in dark and light under all the conditions studied.

Chase incubations (Table II) showed that the [^{3}H]inositol accumulated during preincubation could be chased into PI by addition of unlabeled cytidine; [^{3}H]PI was increased 1.4-fold in dark and 3.5-fold in light, compared to values before the chase incubation. [^{3}H]Inositol was not chased out of [^{3}H]PI by addition of unlabeled inositol alone but could be chased out of [^{3}H]PI during chase incubation in the presence of unlabeled inositol plus cytidine; ^{3}H-labeled PI was reduced by 60% in light and 40% in dark. Total radioactivity in retinas (as a measure of [^{3}H]inositol accumulation) after chase incubations in dark or light was identical ($\sim 27 \times 10^6$ dpm/retina in dark or light). Therefore, availability of [^{3}H]inositol was not a factor in the observed differences in [^{3}H]inositol incorporation into PI.

In retinas preincubated with [^{3}H]cytidine, [^{3}H]cytidine could be chased out of labeled CDG by addition of either inositol or cytidine plus inositol. In the presence of cytidine plus inositol, ^{3}H-labeled CDG content was reduced by 94–98% in light and 64–68% in dark. Total [^{3}H]cytidine content in retinas was identical after chase in dark or light ($\sim 4.20 \times 10^6$ dpm/retina in dark or light).

Autoradiograms (Fig. 2) of retinas preincubated with [^{3}H]inositol and then chase incubated in the presence of unlabeled cytidine (chase-in) in dark (Fig. 2*a*) or light (Fig. 2*b*), or in light with cytidine and inositol (chase-out; Fig. 2*c*), show that, during chase-in in light, radiolabeled grains became concentrated over the outer nuclear layer and the inner segments of photoreceptor cells (Fig. 2*b*), compared with chase-in in dark (Fig. 2*a*) and compared with chase-out incubations in light (Fig. 2*c*). Under chase-out conditions [^{3}H]PI content of retinas was reduced, and radiolabeled grains were only sparsely distributed over the inner segments and were mainly distributed over the outer nuclear, inner nuclear, and ganglion cell layers.

Biochemical analysis of microdissected retinas after chase-in incubations in dark or light (done either in parallel with the ones in Fig. 2, *a* and *b*, or in repetitions of the same experiment) showed a 2.4- to 3.4-fold increase in ^{3}H-labeled PI content within the microdissected photoreceptor cell layer, with mean values (± SD for four determinations) of 0.6 ± 0.1 and 1.7 ± 0.3 pmol incorporated/nmol total phospholipid phosphorus in dark and light, respectively. Within the inner retina layer, [^{3}H]PI content was only slightly higher in light, compared with dark chase-in incubation (0.4 vs. 0.3 pmol/nmol total phospholipid phosphorus).

Incorporation of [^{14}C]glycerol and ^{32}P$_i$ into PI was increased

FIGURE 2 Representative autoradiograms of retinas preincubated with [^{3}H]inositol (1.5 mCi/ml; 0.5×10^{-3} M) in dark and then chase-incubated for 30 min in dark (*a*) or light (*b*) in medium containing unlabeled cytidine (1.5×10^{-3} M) or in light in medium containing unlabeled cytidine plus 10^{-2} M inositol (*c*). Autoradiograms were obtained after 6 d of exposure to photographic emulsion and were either in dark field (*right* side) using unstained sections or in phase contrast (light field; *left* side) using an adjacent section stained with toluidine blue. After chase-incubation in dark (*a*), radiolabeled grains were distributed throughout the outer (*ON*), inner (*IN*) nuclear, and ganglion (*G*) cell layers of the retina. After chase-in in light in the presence of cytidine (*b*) a heavy concentration of radiolabeled grains was noted within the inner segments of photoreceptor cells. The two sets of brackets and dashed line designate the inner segment, outer nuclear and outer plexiform layers, respectively. After chase-out in light, in the presence of cytidine plus inositol (*c*), retinas contained a diffuse distribution of silver grains over the outer and inner nuclear layers. The distribution of radiolabeled grains was sparse over the outer segments (*OS*), outer plexiform (*OP*), inner plexiform (*IP*), and ganglion cell (*G*) layers in dark or light. The density of silver grains over Müller cell processes at the level of the inner limiting membrane (see ganglion cell layer, G; *a–c*) was variable. Bar, 50 μm. × 280.

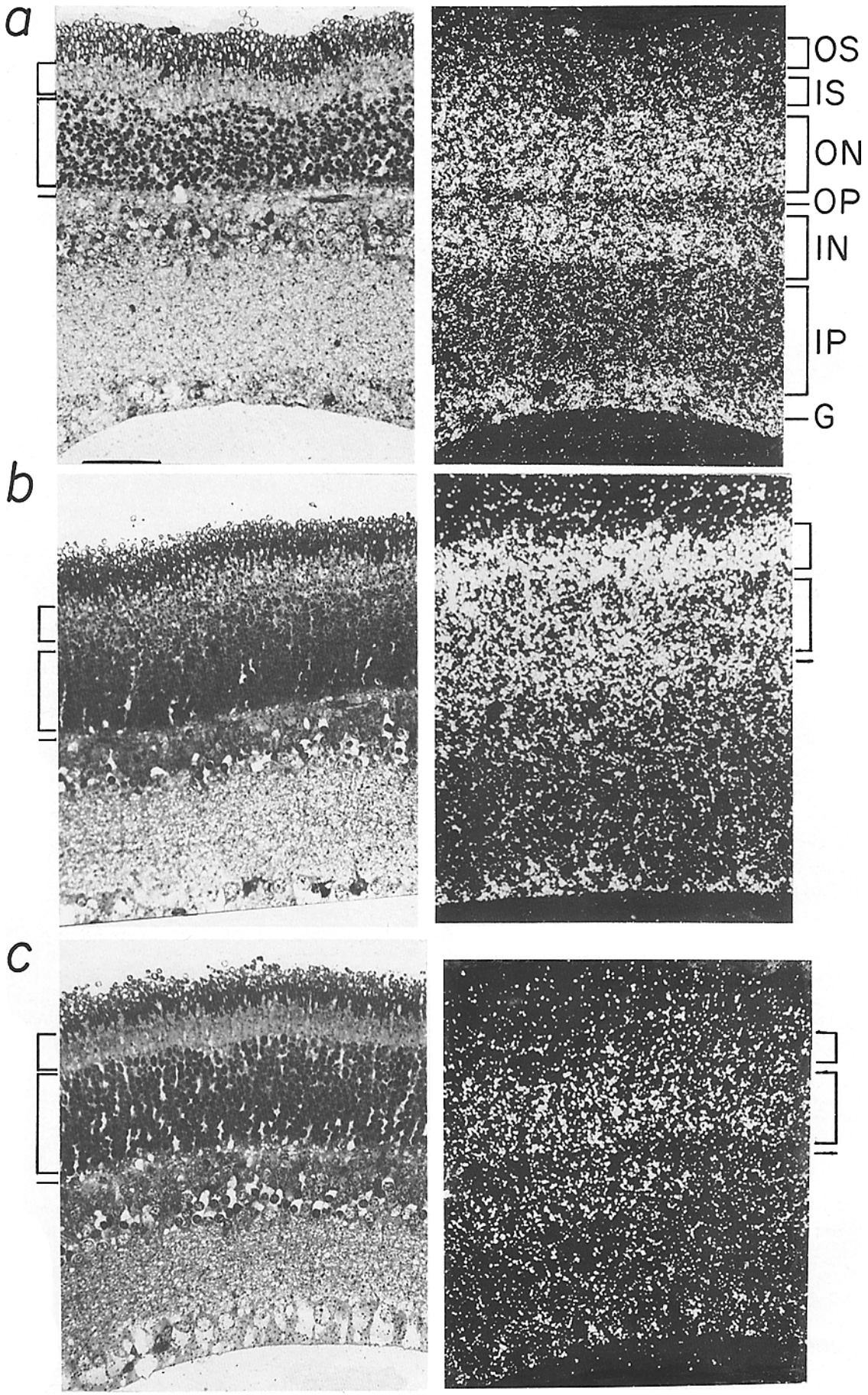
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TABLE II

Effects of Chase Incubation Conditions on [^{3}H]PI or [^{3}H]CDG Content in Retinas Preincubated with [^{3}H]Inositol or [^{3}H]Cytidine

Condition of incubation	Dark	Light
Preincubation, [^{3}H]inositol (0.5×10^{-3} M)	198	—
Chase incubations		
Inositol (10^{-2} M)	210	226
Cytidine (1.5×10^{-3} M)	278	693*
Cytidine (1.5×10^{-3} M) plus inositol (10^{-2} M)	118	76*
Preincubation, [^{3}H]cytidine (10^{-5} M)	3.20	—
Chase incubations		
Cytidine (1.5×10^{-3} M)	3.20	2.20
Inositol (10^{-2} M)	1.55	0.42*
Cytidine (1.5×10^{-3} M) plus inositol (10^{-2} M)	1.09	0.13*

Retinas were preincubated for 30 min in dark with [^{3}H]inositol (1.5 mCi/ml) or [^{3}H]cytidine (250 μCi/ml) at the designated concentrations. Chase incubations were carried out for 30 min in dark or light. The data are expressed as pmol incorporated per retina. Each value represents the mean of six to eight analyses; all standard deviations were within 15–20% of the mean.

* Significantly different from dark value $P < 0.05$ (Student's t test).

TABLE III

Effects of Cytidine plus Inositol on Incorporation of [^{14}C]Glycerol and $^{32}P_i$ Into Lipids in Retinas Incubated in Dark or Light

	Dark		Light	
Precursor	Standard	Enriched	Standard	Enriched
[^{14}C]Glycerol				
PI	5.4	10.7*	12.1	27.3*
Phosphatidic acid	4.0	2.3	7.4	2.4*
CDG	1.3	1.3	1.8	3.0
Polyphosphoinositides	2.5	3.9	3.8	5.1
Sum of remaining phospholipids	4.0	6.6	5.1	10.1*
Neutral lipids	11.9	11.7	15.8	10.3*
$^{32}P_i$				
PI	107	150	186	280*
Phosphatidic Acid	46	49	76	38*
CDG	25	26	36	42
Polyphosphoinositides	346	434	592	773
Sum of remaining phospholipids	12	12	16	24*

Retinas were incubated for 30 min in dark or light with [^{14}C]glycerol (20 μCi/ml; 144 Ci/mmol) and $^{32}P_i$ (orthophosphoric acid 15.6 dpm/10^{-18} mol) either in the standard medium or in the enriched medium (i.e., in the presence of cytidine [1.5×10^{-3} M] plus inositol [10^{-2} M]). Data are expressed as 10^{-12} mol [^{14}C]glycerol incorporated or as 10^{-18} mol $^{32}P_i$ incorporated into lipids on a whole retina basis. Values represent the mean of six to eight analyses; SD were within 15–20% of the mean.

* Significantly different from corresponding standard incubation $P < 0.01$ (Student's t test).

in media supplemented with cytidine plus inositol (enriched medium, Table III). The enriched medium did not have a significant effect on the incorporation of these precursors into other lipids in dark, whereas in light the radioactivity associated with phosphatidic acid and neutral lipids was significantly reduced, and that associated with the sum of remaining phospholipids (particularly phosphatidylcholine and cardiolipin) was increased, compared to values obtained in retinas incubated in the standard medium. The L/D ratios for [^{14}C]PI were 2.2 and 2.7, and those for [$^{32}P_i$]PI were 1.7 and 1.9 in the standard and enriched media, respectively.

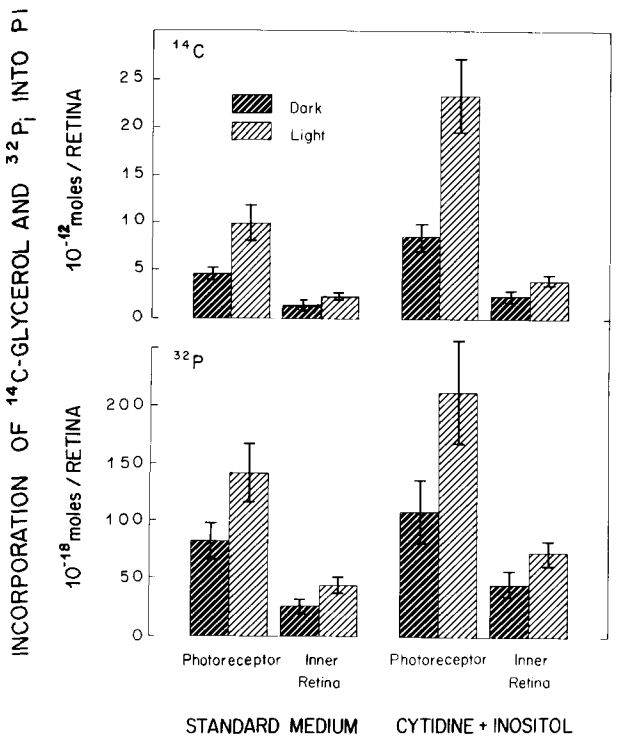

FIGURE 3 Incorporation of [^{14}C]glycerol and $^{32}P_i$ into PI in microdissected photoreceptor cell and inner retina layers, from retinas incubated for 30 min in dark or light in the standard medium or in medium supplemented with cytidine (1.5×10^{-3} M) and inositol (10^{-2} M). The data for PI synthesis were calculated from the fraction of total radioactivity associated with the PI spot and from the total [^{14}C]glycerol or $^{32}P_i$ incorporated on a whole retina basis. Values for the microdissected layers have been normalized so that the sum of corresponding photoreceptor cell and inner retina layers was rendered equivalent to values obtained for whole retinas. The bars represent the mean and the lines within the bars ± SD for four to six analyses.

Microdissection of retinas showed that the effects of light and enriched medium were greatest within the photoreceptor cell layer (Fig. 3). The L/D ratios for [^{14}C]PI within the photoreceptor cell layer were 2.4 and 2.8, and those for $^{32}P_i$ were 1.7 and 2.1 in the standard and enriched media, respectively. In terms of total retinal PI synthesis from [^{14}C]-glycerol and $^{32}P_i$, ~70% was associated with the photoreceptor cell layer, and 30% with the inner retina in dark or light. Within the inner retina as well, light and cytidine plus inositol enhanced the synthesis of PI, and the L/D ratios were close to 1.7 for both precursors.

DISCUSSION

These biochemical and autoradiographic studies show that light-enhanced turnover of PI is associated with the inner segments of photoreceptor cells. Whereas previous studies showed that CDG accumulated in dark within photoreceptor cell inner segments (9), the present studies show that light-enhanced conversion of CDG to PI also occurs mainly within the inner segments of photoreceptor cells. In addition, the present studies provide evidence that two different pools of PI can be distinguished within the photoreceptor cells: one of these associated with photoreceptor cell inner segments has a rapid turnover rate, whereas a second pool of PI associated with membranes in the outer nuclear layer has a slower

turnover rate. In retinas preincubated with [^{3}H]inositol followed by chase incubations with unlabeled cytidine (chase-in), photoreceptor cell inner segments became densely labeled with [^{3}H]PI in light compared with dark, whereas, in chase-out incubations with both cytidine and inositol, [^{3}H]PI within the retina was reduced by 60% in light, and radiolabeled grains were sparse over photoreceptor inner segments but were retained over the outer nuclear layer.

Light-enhanced incorporation of [^{3}H]inositol into PI is shown to occur via a cytidine-dependent pathway, which involved the formation of CDG from CTP and phosphatidic acid and conversion of CDG to PI in the presence of inositol (see Fig. 7 in preceding paper, reference 9). This last reaction, mediated by the activity of CDG:inositol transferase, has been studied in microsomal preparations of guinea pig brain (4) and rat liver (12). In these preparations the K_m values for this enzyme were in the range of $1-2 \times 10^{-3}$ M, close to the observed K_m values for [^{3}H]inositol incorporation into PI in intact rat retinas. The apparent affinity of the reaction of [^{3}H]inositol was higher in light than in dark incubations (a K_m of 0.7×10^{-3} M in light, versus a K_m of 2×10^{-3} M in dark). This increase in affinity may explain at least in part the observation that at low concentrations of [^{3}H]inositol in the medium, its incorporation into PI was enhanced in light, whereas, in the presence of higher ($\geq 10^{-3}$ M) inositol, cytidine was required for this effect to occur. The requirement for cytidine, i.e., CDG as an intermediate in the light-enhanced turnover of PI, was further substantiated in chase incubations (Fig. 2 and Table III). A possible explanation for this requirement is that endogenous cytidine or trace amounts of [^{3}H]cytidine (incubations with labeled cytidine only) were used preferentially for synthesis of RNA in light and, therefore, were limiting for CDG synthesis. The finding that [^{3}H]CDG levels were reduced in retinas incubated in media supplemented with 10^{-2} M inositol further supports this interpretation.

In addition to the above pathway, a cytidine-independent mechanism for [^{3}H]inositol incorporation into PI was observed in dark in the presence of high levels of inositol ($\geq 10^{-3}$ M) in the medium. Such a cytidine-independent pathway, thought to be catalyzed by PI:inositol exchange enzyme (12), has been reported to be the major pathway for hormone-dependent increases in [^{3}H]inositol incorporation into PI in isolated rat liver cells (8). However, in contrast to the studies on liver cells, the present study (Table I) shows that inositol exchange reactions could not sustain light-dependent increases in the incorporation of [^{3}H]inositol into PI.

Both turnover and *de novo* synthesis of PI have been shown to be enhanced in light in rat (6, 10, 11) and toad (2, 3) retinas. Even though PI comprises <5% of total retinal phospholipids (1), its rate of synthesis from labeled glycerol in light has been shown to exceed that of all other phospholipids (3, 11). In the present studies, incorporation of [^{14}C]glycerol and $^{32}P_i$ into PI was increased in light within the photoreceptor cell layer when incubation media were supplemented with both unlabeled cytidine and inositol. In light in the presence of inositol and cytidine, ~50% of the total [^{14}C]glycerol and 25% of $^{32}P_i$ incorporated into lipid were associated with PI within the photoreceptor cell layer. The possible significance of this high rate of PI synthesis within the photoreceptor cells in light is not known at present.

It is concluded from these studies that light-enhanced turnover and synthesis of PI within photoreceptor cell inner segments occur via a cytidine-dependent mechanism. Furthermore, the results suggest that availability of both cytidine and inositol may play a role in the light-dependent changes in PI metabolism.

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