Biosynthesis of molecular species of inositol, choline, serine, and ethanolamine glycerophospholipids in the bovine retina

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Abstract The distribution of radioactivity among molecular species of inositol, choline, serine, and ethanolamine glycerophospholipids was studied in whole bovine retinas and in microsomes from retinas incubated with [^{3}H]glycerol, [^{3}H]inositol, or [^{3}H]serine, in the presence and absence of propranolol. Most of the labeled glycerol was incorporated into docosahexaenoate-containing molecular species (hexaenes and dipolyunsaturates), which suggests that they are synthesized de novo. The largest accumulation of label in these species occurred in phosphatidylinositol, although they were only a minor component of this phospholipid. At short incubation times, these species, as well as monoenes and saturates, incorporated higher percentages of both [^{3}H]glycerol and [^{3}H]inositol than did the tetraenes. Labeling of tetraenes increased thereafter, suggesting that they are produced by acyl-exchange reactions from less unsaturated species. Propranolol was shown to stimulate phosphatidylinositol and polyphosphoinositide synthesis by preferentially enhancing first the labeling of monoenoic and saturated phosphatidylinositols, and subsequently tetraenes. Labeled glycerol was not redistributed among species of phosphatidylcholine with time. Propranolol inhibited the synthesis of monoenes and saturates to a greater extent than other species of phosphatidylcholine. The labeling of diglycerides was first inhibited, and then stimulated by propranolol; tetraenoic diglycerides were the major product accumulated. Propranolol stimulated [^{3}H]serine incorporation into phosphatidylserine and phosphatidylethanolamine, with no alteration in distribution of radioactivity among species; [^{3}H]glycerol incorporation in phosphatidylethanolamine was inhibited, and its incorporation into phosphatidylserine was stimulated. Labeled serine and glycerol were concentrated largely in docosahexaenoate-containing species of phosphatidylethanolamine and phosphatidylserine.—**Aveldaño, M. I., S. J. Pasquare de Garcia, and N. G. Bazán.** Biosynthesis of molecular species of inositol, choline, serine, and ethanolamine glycerophospholipids in the bovine retina. *J. Lipid Res.* 1983. **24:** 628–638.

Supplementary key words propranolol • lipid synthesis • diacylglycerols

Glycerophospholipids are known to display a high degree of molecular heterogeneity in mammalian tissues. In addition to their function as structural components of membranes, some molecular species may participate in specific biophysical and biochemical functions. For example, saturated species of phosphatidylcholine are synthesized and secreted by alveolar type II cells in the lung (1) and are thought to account for the stability and low surface tension of the surface film at the air–liquid interface in alveoli. Arachidonate-containing molecular species, highly concentrated in platelet phospholipids, serve as a source of free arachidonic acid for the synthesis of prostaglandins, thromboxanes, and hydroxyacids (2). Glycerophospholipid molecular species containing large amounts of docosahexaenoate (supraenes) have been described in retina (3, 4). These species, containing mainly polyunsaturated fatty acids in both positions of the glycerol backbone, are highly concentrated in photoreceptor membranes (5, 6). It has been suggested that dipolyunsaturated species of phosphatidylserine (PS) play a role in calcium binding during visual excitation (7). However, knowledge of the function and properties, as well as the composition and biosynthesis, of lipid molecular species in most tissues is still fragmentary.

In this report, the distribution of labeled precursors among molecular species of lipids in retina is described under basal conditions and in the presence of propranolol. Previous work showed that an active de novo synthesis of lipids takes place in the bovine retina in vitro (8) and that propranolol stimulates the labeling of acidic phospholipids (phosphatidylinositol (PI), phosphatidic acid) while depressing that of triglycerides, phosphatidylcholine (PC), and phosphatidylethanolamine (PE) (9–11). Such a redirection of the glycerolipid biosynthetic

Abbreviations: CGP, EGP, SGP, and IGP, choline, ethanolamine, serine, and inositol glycerophospholipids, respectively; PC, PE, PS, and PI, phosphatidylcholine, -ethanolamine, -serine, and -inositol, respectively; TLC, thin-layer chromatography.

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pathway has been described in a variety of tissues, including lymphocytes (12), pancreatic islets (13), iris muscle (14), and pineal gland (15–17). In the experiments described below, the labeling of major molecular species of retinal lipids by [^{3}H]glycerol, [^{3}H]inositol, and [^{3}H]serine was evaluated. The effects of propranolol were studied to ascertain whether the stimulatory (or inhibitory) effects of propranolol on lipid labeling involve selective effects on molecular species.

MATERIALS AND METHODS

Myo[2-^{3}H]inositol (sp act 17.4 Ci/mmol), [2-^{3}H]glycerol (sp act 9.52 Ci/mmol), and [L-^{3}H(G)]serine (sp act 2.76 Ci/mmol), as well as [^{3}H]toluene standard (Omnifluor) and Aquasol-2 were obtained from New England Nuclear (Boston, MA). The phospholipids used as standards for thin-layer chromatography (TLC) and phospholipase C were obtained from Sigma Chemical Co. (St. Louis, MO). Cattle eyes were obtained from a local abattoir and packed in crushed ice for transport to the laboratory. The solvents were usually distilled before use.

Incubations and lipid preparations

Dissection of the eyes was performed on ice in dim light. Retinas were incubated at 37°C in the medium described by Ames and Hastings (18), with 2 mg/ml glucose, under 95% O_2:5% CO_2. After 10 or 20 min preincubation as indicated in pertinent experiments, the radioactive precursors were added (5 μCi/retina), and incubation continued for the periods indicated. In the cases when propranolol (0.5 mM) was studied, it was added at the beginning of preincubation and maintained thereafter. When fresh media was added for reincubation, this contained 0.5 mM propranolol where indicated. Microsomal fractions were obtained as described elsewhere (19), from retinas incubated 30 min in the presence of the labeled precursors with or without 0.5 mM propranolol.

Retinas or microsomes were homogenized in chloroform–methanol (20), and protein was separated by centrifugation. The extracts were partitioned with 0.05% $CaCl_2$ containing unlabeled inositol, glycerol, or serine (0.1%) as appropriate, and washed with Folch's upper phase, also containing the unlabeled carriers. The polyphosphoinositides were extracted from the residues at 37°C with acidified solvents (21).

Thin-layer chromatography

Polyphosphoinositides were isolated on potassium oxalate-impregnated silica gel (22). Other phospholipids were isolated by preparative two-dimensional TLC (23). To compare [^{3}H]inositol and [^{3}H]glycerol incorporation, phosphatidylinositols were subjected to argentation TLC in their intact form (24). Otherwise, inositol glycerophospholipid (IGP), choline glycerophospholipid (CGP), and diacylglycerols were converted to acetyldiglycerides and resolved into molecular species as previously described (6). Serine glycerophospholipid (SGP) and ethanolamine glycerophospholipid (EGP) were converted to trifluoroacetamides, which were purified by TLC and resolved into species according to the method of Yeung et al. (25). Species were located under UV light after spraying with 2′, 7′, dichlorofluorescein (24).

Liquid scintillation counting

Polyphosphoinositides were counted on scrapings from the TLC plates after 1 ml of water and 10 ml of Triton X-100 with 5% Omnifluor in toluene were added. Molecular species were counted after the TLC scrapings were mixed in vials with 1 ml of 2 M NaCl, and 10 ml of Aquasol-2 was added (6). Counting efficiency was determined using [^{3}H]toluene as standard and various amounts of scrapings from argentation TLC plates, sprayed with dichlorofluorescein, as quencher.

Protein and lipid phosphorus

Protein and lipid phosphorus were determined according to the methods of Lowry et al. (26) and Rouser, Fleischer, and Yamamoto (27), respectively.

RESULTS

Phosphoinositides

The time course for the incorporation of labeled glycerol in bovine retina phosphatidylinositol and the stimulatory effect of propranolol on the labeling of this lipid have been described elsewhere (8–11). [^{3}H]Inositol and [^{3}H]glycerol were incorporated in retina PI and polyphosphoinositides (**Table 1**). A 2-fold stimulation of [^{3}H]inositol incorporation into PI was elicited by propranolol throughout the incubation period. After a lag of about 15 min, the labeling of polyphosphoinositides was also enhanced by the drug. The results suggest a precursor–product relationship among phosphoinositides in retina.

The distribution of [^{3}H]glycerol and [^{3}H]inositol among major molecular species of PI after 30 min incubation is shown in **Fig. 1.** Tetraenes concentrated most of the [^{3}H]inositol at 30 min, but this was not the case at earlier incubation times. Thus, at 5 min incu-

TABLE 1. Incorporation of [^{3}H]inositol and [^{3}H]glycerol into bovine retina phosphoinositides in the presence (+) or absence (−) of propranolol

Precursor	Incubation Time	PI		DPI		TPI	
		−[a]	+[a]	−[a]	+[a]	−[a]	+[a]
	min	*dpm/100 mg of protein* × 10^{-3}					
[^{3}H]Inositol	5	24 ± 5	47 ± 1	0.2 ± 0.1	0.2 ± 0.1[b]	0.1 ± 0.03	0.1 ± 0.02[b]
	15	84 ± 12	146 ± 39	0.4 ± 0.1	0.3 ± 0.2[b]	0.3 ± 0.06	0.2 ± 0.03[b]
	30	157 ± 22	328 ± 17	0.9 ± 0.2	3.5 ± 1.0	0.5 ± 0.1	1.0 ± 0.1
	60	295 ± 31	685 ± 112	1.8 ± 0.7	5.1 ± 2.0	0.9 ± 0.4	2.5 ± 0.4
[^{3}H]Glycerol	30	305 ± 43	970 ± 224	2.3 ± 1.0	6.3 ± 2.3	1.8 ± 0.9	4.0 ± 0.8

After 20 min preincubation, the precursors were added, and the retinas were incubated for the specified intervals. Propranolol (0.5 mM) was added at the beginning of the preincubation period and maintained thereafter. PI was extracted with neutral solvents, and di- and triphosphoinositides (DPI and TPI) were extracted with acid solvents.

[a] Values are mean ± SD from three samples.

[b] Nonsignificant differences with respect to controls. The rest of the differences are significant ($P < 0.01$).

bation, 74% of the label was in species more unsaturated than the tetraenes, 26% was in less unsaturated species, while no label was detected in the large tetraenoic fraction (**Table 2**). Radioactivity accumulated rapidly in the latter, and by 30 min 53% of the [^{3}H]inositol was found in tetraenes, in contrast to 35% and 12% in polyenes and oligoenes, respectively (Fig. 1). Because the latter are minor components of retina PI (6), the results given in Table 2 suggest that these species are synthesized at higher rates than are tetraenes.

The distribution of [^{3}H]inositol among major molecular species of PI was altered markedly by propranolol (Fig. 1). At 5 min incubation, the labeling of oligoenes was stimulated 5-fold and that of polyenes 2-fold (Table 2). At 30 min, labeling of both species was stimulated 3-fold, in contrast to labeling in the tetraenoic species, which increased only moderately. This resulted in the alteration of ^{3}H distribution among species shown in Fig. 1. Table 2 shows that the incorporation of [^{3}H]inositol into species of retinal PI involved a time-dependent redistribution of the label in favor of the tetraenes. In the presence of propranolol, however, tetraenes were only slightly stimulated, while the other species were stimulated 2- to 3-fold throughout the whole period of incubation.

Hence, although propranolol stimulated the incorporation of both [^{3}H]inositol and [^{3}H]glycerol into PI, the molecular species labeled by these precursors are not the same. Moreover, based on relative amounts of species in entire retina (6), both precursors are more actively incorporated into polyenes or oligoenes than into tetraenes, both in controls and in propranolol-stimulated retinas.

The distribution of [^{3}H]glycerol among species of microsomal PI is shown in **Table 3.** In this and the following experiment, PI was converted to acetyldiacylglycerols, which, compared to the intact phospholipid, allow better resolution into species by argentation TLC, and improved detection of minor species, because

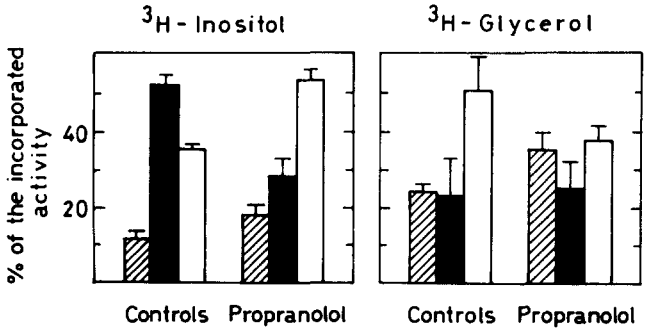

Fig. 1. Distribution of [^{3}H]inositol and [^{3}H]glycerol among oligoenoic (▨), tetraenoic (■), and polyenoic (□) phosphatidylinositols in the presence or absence of propranolol. Retinas were incubated for 30 min with either precursor as detailed in Table 1, and intact PIs were resolved by argentation TLC.

TABLE 2. Incorporation of [^{3}H]inositol and [^{3}H]glycerol into retina phosphatidylinositols in the presence (+) or absence (−) of propranolol

Precursor	Incubation Time	Molecular Species					
		Polyenes		Tetraenes		Oligoenes	
		−	+	−	+	−	+
	min						
[^{3}H]Inositol	5	11	24	0	14	4	19
	15	34	79	35	43	14	23
	30	55	176	83	94	19	62
[^{3}H]Glycerol	30	157	367	70	247	71	343

After 20 min of preincubation, retinas were incubated with the labeled precursors. Propranolol was either present (+) or absent (−) throughout preincubation-incubation. Incorporation values were obtained by multiplying the mean of the percentages of radioactivity incorporated in the species under study by the incorporated activity and dividing by 100.

TABLE 3. Distribution of [^{3}H]glycerol among microsomal phosphatidylinositols in the presence (+) or absence (−) of propranolol

Species	% Distribution of Label		Propranolol-induced Increase, %[b]	Relative Specific Activity[c]	
	− (9)[a]	+ (4)[a]		−	+
Supraenes	12.1 ± 2.2	10.1 ± 1.1	364	4.3	3.6
Hexaenes	29.0 ± 3.6	23.9 ± 1.5	358	5.6	4.6
Pentaenes	7.9 ± 1.8	8.3 ± 1.1	455	1.6	1.7
Tetraenes	28.4 ± 2.2	23.6 ± 0.6	361	0.4	0.3
Trienes	5.3 ± 0.8	5.8 ± 0.6	470	0.8	0.8
Dienes	3.7 ± 0.9	5.3 ± 1.1	621	1.5	2.1
Monoenes	7.0 ± 1.7	13.0 ± 1.2	810	1.9	3.6
Saturates	6.7 ± 2.6	10.0 ± 1.4	653	2.3	3.4
Total incorporated activity[d]	15.6 ± 3.8	67.9 ± 5.1	435		

After 10 min preincubation, retinas were incubated for 30 min with [^{3}H]glycerol, and microsomes were isolated. Propranolol (0.5 mM) was present during preincubation and incubation of intact retinas. PI was converted to acetyldiacylglycerols and species were resolved by argentation TLC.

[a] Values are means ± SD from the number of samples given in parentheses.

[b] Incorporation into species was calculated as % radioactivity in species × total incorporated activity/100.

[c] Calculated as % radioactivity in species/molar % (from Ref. 6) × 100.

[d] Values given as dpm/mg protein × 10^{-3}.

unlabeled acetyldiglycerides can be added as carriers. The incorporation of [^{3}H]glycerol into PI per mg of protein was 5.1-fold higher in microsomes (Table 3) than in whole retina (Table 1). The effect of propranolol on PI synthesis was also greater in microsomes. Table 3 shows that the effect of the drug on the "oligoene" group of Fig. 1 involved mainly monoenes, followed by saturates and dienes. Relative specific activities of species show that hexa- and supraenes were synthesized at the highest rates, and tetraenes at the lowest.

After a short incubation in the presence of [^{3}H]glycerol (10 min), the precursor was removed, and the distribution of label among retina PIs was followed (**Fig. 2**). In controls, the percent label in tetraenes continued to increase after removal of [^{3}H]glycerol, while that of monoenes and saturates began to decline after a short spike. Monoenes and saturates were highly stimulated by propranolol at the beginning of incubation, and the percentage of radioactivity in these species remained higher than in controls throughout the incubation interval. Tetraenes were stimulated later. The time course of the distribution of radioactivity suggests that tetraenes may be synthesized from monoenes and saturates. A surprisingly high and steady synthesis of docosahexaenoate-containing species of PI was seen in control retinas. Hexaenes incorporated most of the label throughout the incubation period. The percent decreases in labeling of these species in the presence of propranolol reflected the more active changes taking place in the other species (Fig. 2 and **Fig. 3**).

Phosphatidylcholines

Monoenes and saturates collectively made up half of the phosphatidylcholines from whole retina (28% and 23%) and microsomes (35% and 17%, respectively) (6). In contrast to phosphatidylinositols, these species of PCs appeared to be synthesized after rather than before other species, as judged from their relative enrichment in radioactivity at 30 min incubation, compared to 10 min (**Table 4**). Hexaenes, which make up 20% of retina PC (6), incorporated most of the radioactivity. At short incubation times, propranolol exerted the greatest effect on the synthesis of hexa- and supraenes; at 30 min incubation, however, the most inhibited species were monoenes and saturates. Both the total incorporated activity and the distribution of radioactivity among species at 30 min incubation with the drug resembled the patterns seen in control retinas at 10 min, which indicates that the late synthesis of monoenes and saturates was impaired by the drug. The slower labeling of monoenes and saturates with respect to hexaenes was also seen after removal of [^{3}H]glycerol (Fig. 3). In the presence of propranolol, virtually no further synthesis of monoenes and saturates took place. Since these species are the most stimulated in phosphatidylinositol and the most inhibited in phosphatidylcholine, with respect to other species, the results indicate that these species are preferentially involved in the effects of propranolol on de novo glycerolipid synthesis. The distribution of radioactivity among microsomal phosphatidylcholines and

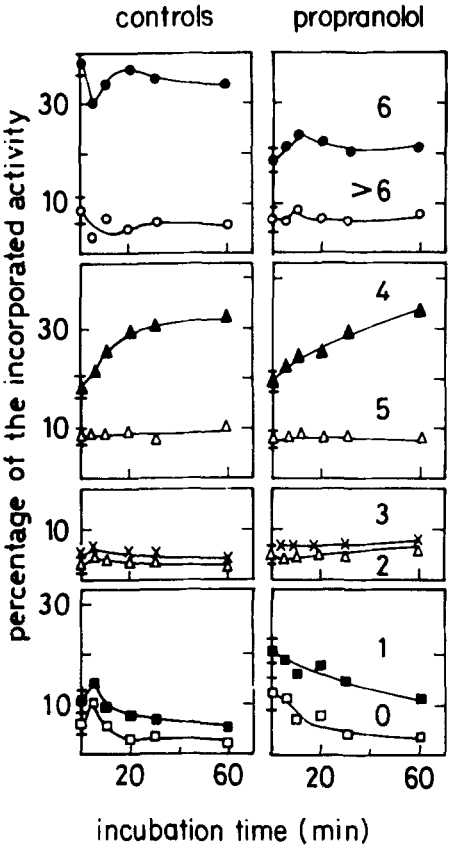

Fig. 2. Effect of propranolol on the distribution of [^{3}H]glycerol radioactivity among phosphatidylinositols during reincubation in glycerol-free media. After 10 min of preincubation, retinas were incubated for 10 min with [^{3}H]glycerol, thoroughly rinsed with fresh medium, and incubated in glycerol-free medium. Propranolol (0.5 mM) was present throughout preincubation, labeling, and reincubation. Acetyldiacylglycerols were prepared and isolated by argentation TLC. The numbers (0 to >6) indicate the number of double bonds per molecule, e.g., 0, saturates, 1, monoenes, 4, tetraenes, >6, supraenes, etc.

the effects of propranolol on molecular species were very similar to findings in whole retina (Table 4). This indicates that most of the effects of the drug on glycerolipid synthesis take place in the endoplasmic reticulum.

Diacylglycerols

The labeling of diacylglycerols increased as a function of time in control incubations in the presence of [^{3}H]glycerol (**Table 5**). When the precursor was removed (Fig. 3), diglycerides were only partially consumed, because part was being utilized for the synthesis of phospholipids and triglycerides, but some was still being synthesized from the remaining phosphatidic acid. Monoenes and saturates were synthesized later than polyenes, as suggested by the distribution of radioactivity at 10 and 30 min (Table 5), and by the lag in their consumption shown in Fig. 3.

A drastic inhibition of diglyceride labeling was elicited by propranolol at short incubation times, which is consistent with the inhibition of PC synthesis and with the hypothesis that the drug is inhibiting phosphatidate phosphohydrolase, inasmuch as these reductions in labeling occurred in the presence of a highly stimulated labeling of phosphatidate (9–11). In spite of the 5-fold decrease in diglyceride labeling at 10 min, the effect did not involve any specific diglyceride species. However, prolonged incubations of retinas with propranolol resulted in an accumulation of labeled diglycerides (9, 10) (Table 5). A significant stimulation of tetraenes occurred among the stimulated diglycerides. The dual effect of propranolol on diglyceride labeling obviously can not be explained solely by inhibition of phosphatidate phosphohydrolase, and suggests that part of the accumulated diglycerides may originate from sources other than de novo synthesis. Diacylglycerols also accumulated when [^{3}H]glycerol was removed after a short incubation (Fig. 3).

Serine and ethanolamine glycerophospholipids

The distribution of [^{3}H]glycerol among serine and ethanolamine glycerophospholipids was studied after their conversion to trifluoroacetamides, first to allow comparison with the distribution of [^{3}H]serine, and second because CDP-diacylglycerol and phosphatidylglycerol were found to partially overlap PS and PE spots, respectively, in our preparative TLC separations. The trifluoroacetamides were purified by TLC, which allows separation of the contaminant lipids.

More than 80% of the [^{3}H]glycerol accumulated in docosahexaenoate-containing molecular species of EGP after 30 min incubation (**Fig. 4**), and was distributed almost equally between supraenes and hexaenes. Since these species represent 17% and 53% of the retina PEs, respectively (6), a more active de novo synthesis of supraenes than hexaenes is suggested. Although propranolol inhibited the labeling of PE 8-fold, no selective effects on molecular species were detected.

Ethanolamine glycerophospholipids also incorporated a small amount of [^{3}H]serine after 30 min incubation (Fig. 4). Hexaenes and polyenes incorporated most of the label. Incorporation of [^{3}H]serine in this lipid was stimulated by propranolol, unlike that of [^{3}H]glycerol. No significant differences in serine distribution among species with propranolol treatment were found.

[^{3}H]Glycerol was also incorporated into PS (**Fig. 5**), although in very small proportions. Propranolol did not inhibit [^{3}H]glycerol labeling of PS, as it did with PE and PC, but rather produced a slight stimulation. The opposite effect of the drug on these phospholipids suggests

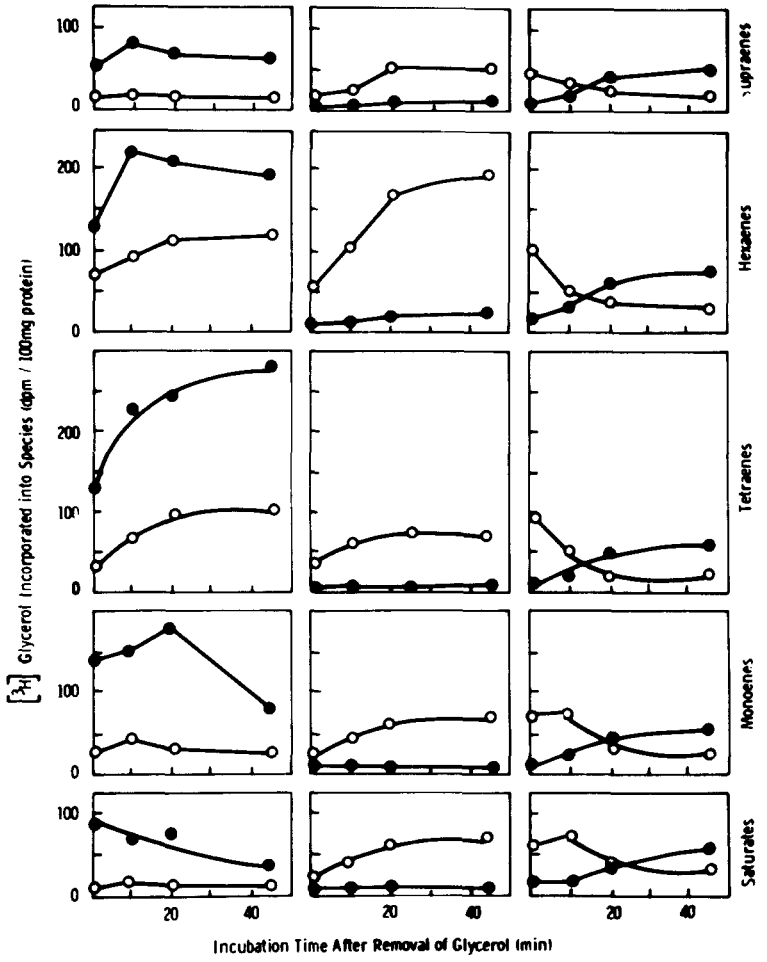

Fig. 3. [^{3}H]Glycerol-labeling of molecular species of retina PI (left), PC (middle), and diacylglycerols (right) in the presence (●), or absence (○) of propranolol. After 10 min preincubation, retinas were incubated 10 min with [^{3}H]glycerol, rinsed with fresh medium, and reincubated in glycerol-free medium. Propranolol (0.5 mM) was present throughout preincubation, labeling, and reincubation. Acetyldiacylglycerols were prepared and resolved by argentation TLC. Incorporation into species was calculated as % incorporated into species × total incorporated activity/100.

that SGP may be synthesized by routes other than base exchange with EGP or CGP. [^{3}H]Glycerol was incorporated predominantly in supraenoic PS (Fig. 5), and propranolol slightly stimulated the labeling of these species. Supraenes are highly concentrated in rod outer segment PS, but represent only 15% of the PS from whole retina and microsomes (6). [^{3}H]Serine was incorporated predominantly in hexaenoic PS. Propranolol stimulated the labeling of PS 5-fold with no significant changes in the distribution of [^{3}H]serine among species.

Most of the [^{3}H]serine incorporation taking place in retinal PE and PS, as well as the stimulatory effect of propranolol on the labeling of both lipids, takes place predominantly in microsomes (11). Propranolol did not alter the distribution of [^{3}H]serine among microsomal PE and PS (**Fig. 6**). A higher percentage of [^{3}H]serine was found in species less unsaturated than tetraenes in PS and PE from microsomes (Fig. 6), compared to whole retina (Figs. 4 and 5).

DISCUSSION

An active de novo biosynthesis of docosahexaenoate-containing molecular species of all major glycerophospholipids in retina is suggested by the high percentages of [2-^{3}H]glycerol incorporated. For the first time, the labeling of hexaenoic and dipolyunsaturated species has been studied separately. Surprisingly, the highest specific activities of [2-^{3}H]glycerol incorporation into these species was in phosphatidylinositol, of which they are minor components (6). A significantly high labeling of species more unsaturated than the tetraenes was also observed in the brain in vivo (28). These species were

TABLE 4. Distribution of [^{3}H]glycerol among phosphatidylcholines in the presence (+) or absence (−) of propranolol

	Incubation Time in Minutes (n)[a]					
	Whole Retina				Microsomes	
	10 (4)		30 (3)		30 (5)	
Species	−	+	−	+	−	+
	% of incorporated activity					
Supraenes	9.3 ± 0.6	6.6 ± 1.2[b]	5.9 ± 1.0	8.5 ± 1.4[b]	8.1 ± 1.4	9.9 ± 1.4
Hexaenes	30.2 ± 1.9	23.6 ± 3.7[b]	27.1 ± 2.0	30.2 ± 2.9	24.2 ± 1.4	29.3 ± 1.5[b]
Pentaenes	8.1 ± 1.1	5.9 ± 1.3	5.2 ± 0.1	6.5 ± 0.5	6.2 ± 0.7	7.2 ± 0.9
Tetraenes	13.9 ± 2.1	13.3 ± 2.7	9.5 ± 0.3	12.7 ± 0.4[b]	11.6 ± 2.2	11.8 ± 1.8
Trienes	3.9 ± 0.5	5.6 ± 0.7	2.4 ± 0.02	2.8 ± 0.1	6.0 ± 0.7	6.8 ± 1.1
Dienes	7.2 ± 0.6	11.1 ± 2.8	9.1 ± 0.2	7.2 ± 0.7	8.1 ± 0.9	7.5 ± 0.6
Monoenes	17.6 ± 1.7	19.4 ± 3.0	24.1 ± 1.4	19.1 ± 1.5[b]	21.5 ± 0.9	16.0 ± 0.9[b]
Saturates	10.4 ± 1.2	13.9 ± 3.1	16.4 ± 1.1	12.5 ± 1.1[b]	14.4 ± 1.1	11.2 ± 1.1[b]
Total incorporated activity[c]	194 ± 28	13 ± 2	953 ± 88	145 ± 8	3468 ± 580	360 ± 50

After 10 or 20 min preincubation, retinas were incubated for the specified intervals in the presence of [^{3}H]glycerol. Microsomes were isolated after 10 min of preincubation and 30 min of incubation of intact retinas in the presence of [^{3}H]glycerol. Propranolol (0.5 mM) was present during preincubation and incubation. Phosphatidylcholine species were separated by argentation TLC of acetyldiacylglycerol derivatives.

[a] Each set of experiments was done separately. Values are means ± SD of the number of samples given in parentheses.

[b] Significant differences with respect to controls ($P < 0.05$).

[c] Values given as dpm/100 mg protein × 10^{-3}. All differences with respect to controls are significant.

also labeled rapidly with [^{3}H]inositol. Tetraenes, the major molecular species of this lipid, were synthesized from both precursors at relatively slower rates. The time course of their labeling suggests that they may be synthesized from other molecular species by acyl-exchange reactions using arachidonate, as may occur in liver (29–33) and brain (28, 32). Monoenoic, saturated, and dienoic species of phosphatidic acid may be used preferentially for the de novo synthesis of PI, these molecular species subsequently serving as substrates for

TABLE 5. Distribution of [^{3}H]glycerol among retinal diacylglycerols in the presence (+) or absence (−) of propranolol

	Incubation Time in Minutes (n)[a]			
	10 (4)		30 (3)	
Species	−	+	−	+
	% of incorporated activity			
Supraenes	11.2 ± 2.1	9.8 ± 2.0	14.4 ± 2.7	7.5 ± 1.8[b]
Hexaenes	23.3 ± 1.1	20.6 ± 2.4	18.9 ± 2.2	23.1 ± 0.9[b]
Pentaenes	5.9 ± 0.5	5.8 ± 0.6	5.0 ± 0.1	6.8 ± 0.4[b]
Tetraenes	19.0 ± 0.7	21.7 ± 2.4	8.8 ± 0.3	14.1 ± 0.1[b]
Trienes	4.5 ± 1.9	6.6 ± 1.6	2.9 ± 0.2	3.5 ± 1.3
Dienes	3.6 ± 1.9	5.6 ± 0.8	5.5 ± 0.5	5.8 ± 0.5
Monoenes	15.6 ± 1.1	13.1 ± 1.2	18.3 ± 1.4	19.0 ± 1.3
Saturates	15.0 ± 0.7	12.6 ± 1.8	25.3 ± 1.2	19.2 ± 2.5[b]
Total incorporated activity[c]	432 ± 30	78 ± 8	1195 ± 87	2249 ± 496

Each set is the result of a separate experiment. Diacylglycerols were isolated from the experiments with whole retinas described in Table 3. Acetyldiacylglycerols were prepared and species were resolved by argentation TLC.

[a] Values are mean ± SD of the number of samples given in parentheses.

[b] Significant differences with respect to controls ($P < 0.05$).

[c] Values given as dpm/100 mg protein × 10^{-3}.

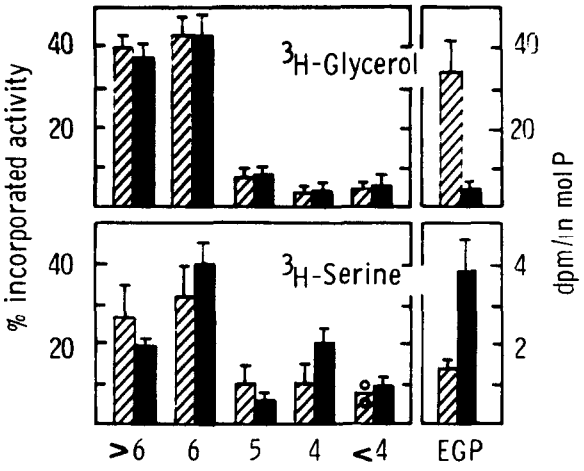

Fig. 4. Incorporation of [^{3}H]glycerol and [^{3}H]serine in ethanolamine glycerophospholipids (EGP). After 10 min of preincubation, retinas were incubated for 30 min with either precursor. Propranolol (0.5 mM) was present during preincubation and incubation. EGP species were resolved as trifluoroacetamides. ▧, Controls; ■, propranolol.

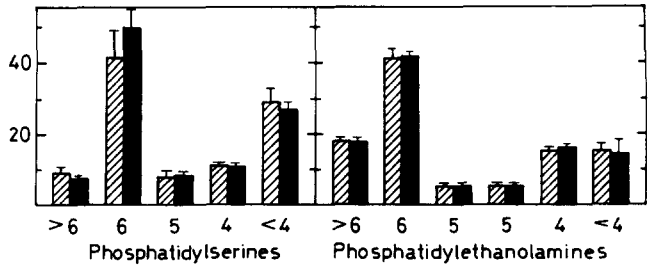

Fig. 6. Distribution of [^{3}H]serine among species of PS and PE from retinal microsomes. After 10 min preincubation, retinas were incubated for 30 min with [^{3}H]serine and microsomes were isolated. Propranolol (0.5 mM) was present during preincubation and incubation. Species were resolved as trifluoroacetamides. ▧, Controls; ■, propranolol.

acyl-transferases. This is supported by the effect of propranolol, which selectively enhances the labeling of these species when the de novo biosynthesis of PI is stimulated. The time-course effect of propranolol was also consistent with this hypothesis, i.e., the stimulation of monoenes and saturates preceded that of tetraenes.

The labeling of tetraenoic PI by [^{3}H]inositol was more active than labeling by [^{3}H]glycerol, as suggested by the distribution of both precursors after 30 min incubation. Part of the [^{3}H]inositol may be incorporated by de novo synthesis, but part is likely to originate in turnover of the polar moiety, i.e., exchange of labeled for unlabeled inositol. Although the contribution of these reactions cannot be ascertained from the present data, the turnover of the polar moiety of tetraenoic PI is probably more active than that of other species. The distribution of [^{3}H]serine among molecular species of PS and PE also differed from that of [2-^{3}H]glycerol and the differences are indicative of the specificity of the different enzymes involved in the replacement of the polar moiety and de novo synthesis, respectively.

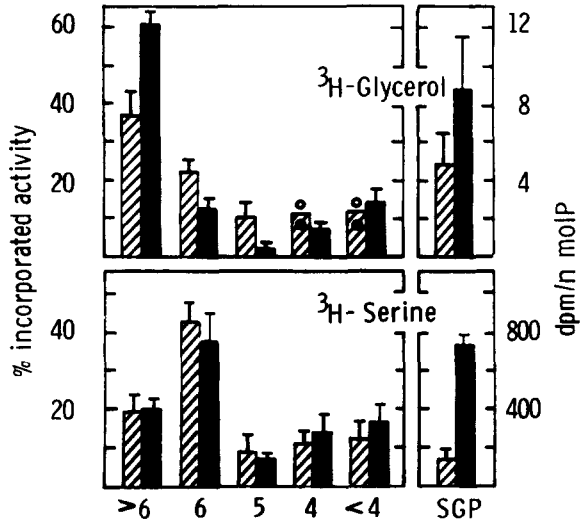

Fig. 5. Incorporation of [^{3}H]glycerol and [^{3}H]serine in serine glycerophospholipids (SGP). SGP were obtained as described in Fig. 4 for EGP. ▧, Controls; ■, propranolol. Incubation as in Fig. 4.

In retinas incubated with [2-^{3}H]glycerol, phosphatidylinositol rapidly attains the highest specific activity among glycerophospholipids, and its synthesis precedes that of PC, PE, and triglycerides (8–11). All the labeling experiments using this precursor apply only to glycerophospholipids synthesized from glycerol-3-phosphate and exclude any lipid synthesized from dihydroxyacetonephosphate. This study shows that labeling of di- and triphosphoinositides, minor components of retinal lipids, also occurred with [^{3}H]glycerol and [^{3}H]inositol. The time course of their labeling and of the stimulation by propranolol of the incorporation of both precursors suggested a precursor–product relationship among phosphoinositides in retina.

In contrast to PI, the pattern of radioactivity distribution among phosphatidylcholines did not show preferential enrichment of a particular molecular species with time. However, hexa- and supraenoic species of PE incorporated nearly 80% of the [^{3}H]glycerol in 30 min incubation, indicating that docosahexaenoate-containing diglycerides may be used preferentially to synthesize PE, as observed in other tissues (34–36). Monoenoic and saturated species of PC in the retina appear to be synthesized at relatively slower rates than the rest of the species, considering that they are major components of PC. The fact that, in the presence of propranolol, monoenes and saturates are the most stimulated species of PI and the most inhibited species of PC indicates a common metabolic origin, and suggests an important role for these species in the de novo synthesis of retinal lipids.

Although the role of individual molecular species of phosphatidic acid has not yet been established, results from parallel experiments demonstrated a significant accumulation of this lipid in retinal microsomes of propranolol-treated retinas, consistent with the hypothesis that the drug inhibits phosphatidate phosphohydrolase (37). Studies of fatty acid composition revealed that palmitic and oleic acids undergo the highest percent increases (37), which again suggests an important role for monoenes and saturates in terms of the effects of the drug. However, no selective effects on molecular species of diacylglycerols were seen in our work after 10 min incubation, when the labeling of diglycerides was inhibited. At 30 min, when the labeling of diglycerides was stimulated, tetraenes were the most stimulated species.

The origin of the stimulated diglycerides remains unclear. Even assuming that their production from phosphatidic acid was inhibited, many other reactions, directly or indirectly influenced by propranolol, may have contributed to this pool. Acylation of monoacylglycerols could be an alternative biosynthetic route switched on under conditions of depressed diglyceride synthesis. However, diglyceride accumulation occurred also when glycerol was removed (Fig. 3). The breakdown of newly synthesized phospholipids may be another possible source of labeled diglycerides. A metabolic cycle relating phosphatidate, phosphoinositides, and diacylglycerols has been proposed to explain the increased turnover of PI elicited by a variety of stimuli, including pharmacological ones (38–40). The reversal of the choline phosphotransferase-catalyzed reaction, which is known to degrade PC in the presence of cytidine monophosphate (41, 42), could be another source of diglycerides, inasmuch as CMP is probably produced in increased amounts due to the stimulated synthesis of PI. However, unless a specific action on newly synthesized molecules of PC were involved, this would not explain either the high levels of radioactivity in the accumulating diacylglycerols or the main role of tetraenoic species in this effect. Thus, inhibition of phosphatidic acid phosphohydrolase may be a primary biochemical effect of the presence of propranolol, but several other reactions may be affected also through mechanisms yet to be determined (e.g., alterations in intracellular calcium levels, ATP concentrations, etc.).

The stimulation of [^{3}H]serine incorporation in PS and PE is an example of other reactions, apart from de novo synthesis, altered by propranolol. Thus, the labeling of PS by [^{3}H]serine was stimulated severalfold in contrast to only a slight increase in the incorporation of [^{3}H]glycerol. Also, while [^{3}H]glycerol incorporation in PE was inhibited, that of [^{3}H]serine was stimulated. Propranolol has been shown to increase the synthesis of SGP by stimulating the Ca^{2+}-catalyzed base-exchange reaction (14). [^{3}H]Glycerol in SGP may conceivably originate in labeled EGP and CGP by base-exchange with unlabeled, endogenous serine. However, the contribution of these reactions to the [^{3}H]glycerol labeling of SGP must be small because of the opposite effects of propranolol on phosphatidylserine on one hand, and on phosphatidylcholine and ethanolamine on the other. [^{3}H]Serine was mainly incorporated in hexaenoic species of PS and PE, while [^{3}H]glycerol was predominantly taken up by supraenoic species in both lipids.

The Ca^{2+}-dependent exchange of labeled L-serine for ethanolamine (or choline) using EGP (or CGP) as lipid substrates is the best known pathway for SGP synthesis in mammalian tissues and takes place exclusively in microsomes (43–46). Previous work proved that such an exchange occurs also in the retina and is stimulated by Ca^{2+} and propranolol (11).

In contrast to [^{3}H]glycerol, [^{3}H]serine labeling of EGP was stimulated by propranolol (Fig. 4). The labeling of PE with [^{3}H]serine has been proposed to arise from decarboxylation of [^{3}H]serine-labeled SGP (11, 47). Although phosphatidylserine decarboxylase is located primarily in mitochondria (46, 48), [^{3}H]-labeled EGP was obtained in microsomes from retinas incubated with [^{3}H]serine (11, 47) (Fig. 6).

In conclusion, monoenes and saturates seemed to play a predominant role in the de novo pathway of acidic phospholipid synthesis, subsequently serving as substrates for the synthesis of tetraenes. Stimulated synthesis of PI (and inhibited synthesis of PC by propranolol) was accompanied by preferential stimulation (or inhibition) in the labeling of monoenes and saturates. Docosahexaenoate-containing species (supraenes and hexaenes) rapidly concentrated high levels of radioactivity in all glycerophospholipids, which suggests that they are synthesized de novo. These species incorporate high levels of [^{3}H]inositol, and [^{3}H]glycerol in PI, and [^{3}H]serine and [^{3}H]glycerol in PE and PS. Hexaenes and supraenes, important components of retinal phospholipids, are probably incorporated into retinal membranes without further modifications.

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