Phospholipids in *Drosophila* Heads: Effects of Visual Mutants and Phototransduction Manipulations

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A procedure was developed to label phospholipids in *Drosophila* heads by feeding radioactive phosphate ($^{32}P_i$). High-performance thin-layer chromatography showed label incorporation into various phospholipids. After 24 h of feeding, major phospholipids labeled were phosphatidylethanolamine (PE), 47%; phosphatidylcholine (PC), 24%; and phosphatidylinositol (PI), 12%. *Drosophila* heads have virtually no sphingomyelin as compared with mammalian tissues. Notable label was in ethanolamine plasmalogen, lysophosphatidylethanolamine, lysophosphatidylcholine and lysophosphatidylinositol. Less than 1% of the total label was in phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate. Other lipids labeled included phosphatidylserine, phosphatidic acid and some unidentified lipids. A time course (3–36 h) study revealed a gradual decrease in proportion of labeled PI, an increase in proportion of labeled PC and no obvious change in labeled PE. There were no significant differences in phospholipid labeling comparing the *no* receptor *p*otential *(norpA)* visual mutant and wild type under light *vs.* dark conditions. However, overall ^{32}P labeling was higher in the wild type fed in the light as compared to the dark and to *norpA* either in light or dark. This suggests that functional vision facilitates incorporation of label. Differences in phospholipid labeling were observed between young and aged flies, particularly in lysophospholipids and poly-PI, implicating phospholipase A_2 function in recycling. Manipulations such as the *o*uter *r*habdomeres *a*bsent and *ey*es *a*bsent mutants and carotenoid deprivation failed to yield notable differences in phospholipid labeling pattern, suggesting that phospholipids important to vision may constitute only a minor portion of the total labeled pool in the head.
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Photoreceptor membranes in *Drosophila* undergo constant turnover in order to reduce the hazards of light exposure (1–3). Such maintenance involves renewal of the major membrane protein, opsin (4), as well as lipids. In the fly, phospholipids in the photoreceptor membrane are known to play a role in signal transduction (5). In *Limulus,* receptor stimulation of hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C (PLC) was shown to result in the release of inositol trisphosphate (IP_3) which, in turn, serves as a second messenger for intracellular calcium mobilization (6–9). The *Drosophila* mutant *norpA* has *no* receptor *p*otential because of a defect in phospholipase C (10,11). *NorpA* is a PLC gene which has been cloned (12) and shows homologies to that in bovine brain (13).

Little is known regarding the distribution and metabolic activity of the phospholipids in *Drosophila* and variances in different mutants. This study describes a novel procedure for labeling the membrane phospholipids in the heads of normal, mutant and carotenoid deprived *Drosophila.* An improved high-performance thin-layer chromatography (HPTLC) procedure (14) allowed the analysis of the phospholipids that were labeled. The labeling protocol was used to study the effects of light and dark, mutants and carotenoid deprivation on membrane phospholipids. Brief reports of some of these findings have been published (15–17).

MATERIALS AND METHODS

Animals. Stocks of *Drosophila melanogaster* were raised at room temperature under cyclic lighting on a standard diet adequate for visual photoreceptor development (18). In the medium of agar, brewers yeast, molasses and cornmeal, the most relevant features were yellow cornmeal and a supplement of β-carotene (0.13 mg/mL) as sources of vitamin A. Carotenoid deprivation was achieved using a medium with defined components, Sang's medium (19), and the control here involved supplementing Sang's food with β-carotene at 0.13 mg/mL. Wild type (Oregon-R) and *w*hite *(w)* were used as normal (red-eyed) and white-eyed controls, respectively. Our red-eyed *norpA* was $norpA^{EE5}$, an effective allele (20) originally from Seymour Benzer at the California Institute of Technology (Pasadena, CA). For white-eyed *norpA,* and to provide a comparison allele also known to be effective (3,21), a stock of $norpA^{P24}$//C[1]Dx *y f; cn bw* (*cin*nabar *brow*n) was constructed and supplied by Mitchell Dushay of Jeffrey Hall's laboratory at Brandeis University (Waltham, MA). Because only males had the *norpA* genotype, the females being heterozygous, only males were used for the mutant and control *(w)* flies in this experiment. We used the electroretinogram to verify our *norpA* stocks. An allele of *ey*es *a*bsent *(eya)* which lacks the compound eyes but not the simple eyes (ocelli) was obtained from Terry McGuire at Rutgers University (New Brunswick, NJ). The well studied *o*uter *r*habdomeres *a*bsent *(ora)* mutant (22,23) selectively deletes the outer rhabdomeres (R1-6), the predominant photoreceptor type in the compound eye. For *ora,* flies were aged in cyclic lighting one week or more because this manipulation insured a better deletion of the rhabdomeres

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Abbreviations: *cn bw, cin*nabar *brow*n; DG, diacylglycerol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-*bis*(β-aminoethyl ether) *N,N,N′,N′* tetraacetic acid; *eya, ey*es *a*bsent; GC, gas chromatography; HPTLC, high-performance thin-layer chromatography; IP_3, inositol trisphosphate; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; NL, neutral lipids; *norpA, no* receptor *p*otential; *ora, o*uter *r*habdomeres *a*bsent; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PE_{pl}, ethanolamine plasmalogen; P_i, inorganic phosphorus; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PS, phosphatidylserine; TG, triglyceride; TLC, thin-layer chromatography; UN, unknowns; *w, w*hite.

(23). The wild type controls in the *ora* experiments were similarly aged.

^{32}P incorporation and extraction. Flies were transferred into foam stoppered glass vials that contained three circles of filter paper (Whatman No. 3) soaked with 30–50 μCi of ^{32}P-inorganic phosphorus (P_i) in the form of Na_2HPO_4 (New England Nuclear, Wilmington, DE) added to 200 μL of 0.32 M sucrose solution. This amount was adequate for a 30-head sample when flies were fed for 24 h. Flies were fed either in the light or in the dark—dark vials were covered with black tape and light ones with translucent tape. Vials were placed near a fluorescent lamp under a humidified glass dish. Vials were plunged into liquid nitrogen and, when the flies were frozen, vials were shaken and rapped vigorously. By this procedure, heads were well separated from bodies and were sorted quickly with a brush at room temperature. These heads were then put into 1 mL of ice-cold 0.32 M sucrose with 50 mM Tris-HCl buffer (pH 7.4) and ethylenediaminetetraacetic acid (EDTA) (1 mM) and homogenized in a glass homogenizer tube fitted with a motor-driven teflon pestle. Lipids were extracted by adding 4 vol of chloroform/methanol (2:1, vol/vol), to the homogenate, followed by vortexing. After centrifugation at 1500 rpm for 5 min, the organic phase was removed and transferred into an evaporating cup. For more complete extraction of acidic phospholipids, a second extraction was performed with 2 vol of acidic chloroform/methanol/12 N HCl (2:1:0.0125, vol/vol/vol), and the organic phase was neutralized with one drop of 4 N NH_4OH before transferring to the evaporating cup. The extract was evaporated to dryness and resuspended in a small volume of chloroform/methanol (2:1, vol/vol). Aliquots were taken for counting of radioactivity using a Beckman LS5800 liquid scintillation counter (Beckman Instrument, Fullerton, CA).

High-performance thin-layer chromatography (HPTLC). For separation of phospholipids, samples were spotted onto 10 × 10 cm Silica gel 60 HPTLC plates (E. Merck, Darmstadt, Germany) which had previously been dipped in a solution containing 1% potassium oxalate and 2 mM EDTA and diluted with methanol in a ratio of 3:2 (vol/vol). The procedures for separation of phospholipids were described by Sun and Lin (14). Plates were developed in one dimension in a solvent system containing chloroform/methanol/acetone/29% ammonium hydroxide (70:40:10:10, by vol). After development, plates were dried and developed again in the same dimension with a solvent system containing chloroform/methanol/29% ammonium hydroxide/water (72:56:4:12, by vol). The second solvent system was specifically used to move PIP and PIP_2 from the origin. In some experiments, where poly-PI (phosphatidylinositol) separation was not deemed necessary, this solvent system was omitted. After development in the second solvent system, plates were exposed to HCl vapor for 3 min to hydrolyze the alkenyl groups of plasmalogen (24). After drying, plates were turned 90° and developed in a third solvent system containing chloroform/methanol/acetone/acetic acid/0.1 M ammonium acetate (70:30:27.5:2.25:5, by vol). After removal of organic solvent, plates were exposed to iodine vapor for visualization. Furthermore, plates were exposed to Kodak (Rochester, NY) X-omatic AR film for autoradiography of the samples. The phospholipid fractions were scraped into scintillation vials for measurement of radioactivity.

RESULTS

Figure 1 shows a typical autoradiogram and identifies the phospholipids extracted from 75 heads after exposure to $^{32}P_i$ for 26 h. The figure shows incorporation of $^{32}P_i$ into minor as well as major phospholipid components. We have consistently observed a spot to the right side of phosphatidylcholine (PC) on the TLC (thin-layer chromatography) plate (PC_r). Analysis of the fatty acid composition of this lipid fraction indicated a higher content of saturated fatty acids (14:0 and 16:0) and a lower content of 18:1 and 18:2 than in PC. Therefore, PC_r is probably a subspecies of PC. We also have observed two labeled lipid fractions to the right of phosphatidylethanolamine (PE). These spots seem to correspond to phosphatidylglycerol and cardiolipin, but the fractions were not firmly identified. Unlike mammalian tissues, practically no sphingomyelin, cerebrosides or ethanolamine plasmalogen (PE_{pl}) were detected in these samples. On the other hand, notable amounts of lysophospholipids were found.

Data in Figure 2 show the phospholipid labeling profiles in *norpA* and wild type, and compare feeding in light *vs.* dark for 24 h. Results showed no major differences in phospholipid distribution between *norpA* and wild type or between light and dark. The distributions of label in PE and PC, as determined from these and many other experiments, are around 47 and 24%, respectively. PC_r

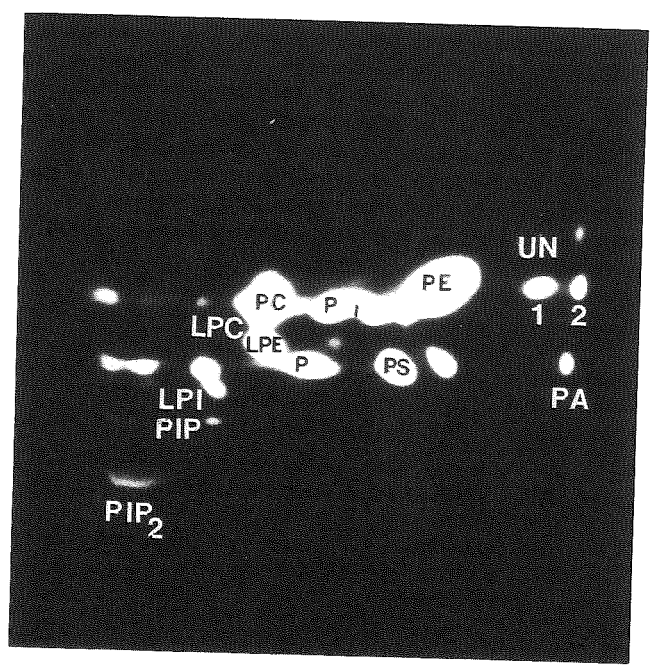

FIG. 1. Autoradiogram of a typical thin-layer chromatography separation of the lipids from *Drosophila melanogaster* heads developed in three solvent systems (ref. 14). The autoradiogram is slightly overexposed to reveal also those phospholipids which are present in small quantities. PIP_2, phosphatidylinositol 4,5-bisphosphate, PIP, phosphatidylinositol 4-phosphate; LPI, lysophosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; PC_r, spot to the right of phosphatidylcholine: PE, phosphatidylethanolamine; PS, phosphatidylserine; and PA, phosphatidic acid; UN 1 and 2 are unknown fractions also presented in Figures 2 and 4; they may correspond to the cardiolipins.

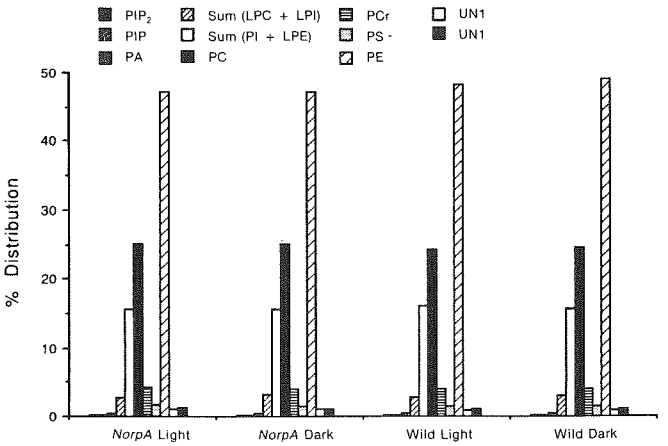

FIG. 2. The percentage distribution of ^{32}P radioactivity in major phospholipids in the heads of the flies studied (*norpA vs.* wild-type, light *vs.* dark reared). Data are pooled from 9 groups of samples (each consisting of 30 heads) in 4 experiments for *norpA* and 7 samples in 3 experiments for wild type. Variations within groups normally do not exceed 5%. Abbreviations as in Figure 1.

comprised 4% of the total radioactivity. In some experiments, the PI spot and the lysoPE (LPE) spot were not clearly separated, and the counts were combined. Nevertheless, on the basis of the plates with good separation, PI was found to comprise 11% and LPE only 2%. Other lysophospholipids, such as lysoPC (LPC) and lysoPI (LPI), comprised 1.6 and 1.4% of total radioactivity, respectively. Other minor phospholipids showing the label are: phosphatidic acid (PA), 0.4%; PIP_2, 0.2%; PIP, 0.2%; phosphatidylserine (PS), 1.6%; unknowns $(UN)_1$, 2.5% and UN_2, 1.1%. Although PS was only labeled sparingly, this phospholipid was a major phospholipid in mass.

We used information from seven experiments to assess incorporation of $^{32}P_i$ into the phospholipids of wild type and *norpA* under light and dark conditions (Fig. 3). Ratios

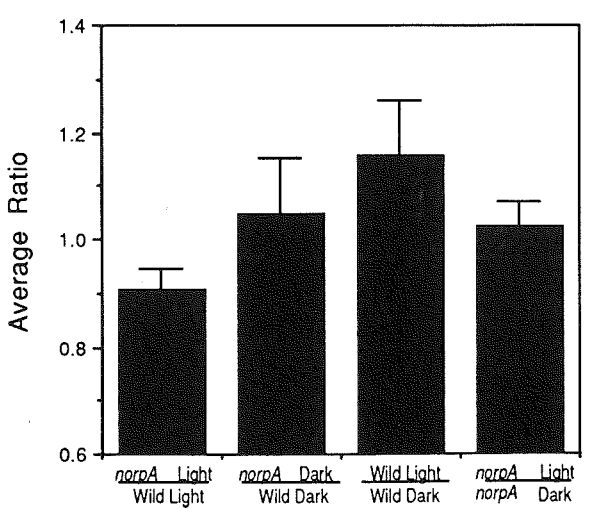

FIG. 3. Overall incorporation of ^{32}P into the phospholipids. Data are based on: (i) the overall counts from aliquots of sample; and (ii) the sum of radioactivity of the lipids on the plates. Data were normalized for the number of fly heads used and the amount of ^{32}P fed. Ratios were calculated using 17 values ± SE except for *norpA* light/*norpA* dark, which was from 19 values ± SE. *NorpA, no* receptor potential.

of about 1 are shown for *norpA*-dark over wild-dark and for *norpA*-light over *norpA*-dark, suggesting that the mutation has no effect in the dark and that the light/dark manipulation has no effect in the mutant. A ratio of about 1.15 was found for wild-light over wild-dark, and the ratio was about 0.9 for *norpA*-light over wild-light. These ratios tend to show that wild type flies have more feeding activity resulting in more labeling in the light, but no dark/light differences are shown for *norpA*, probably because these flies are blind.

When flies were presented with the $^{32}P_i$ for 4 d, rehydrating the filter paper with sucrose but not ^{32}P, the amount of radioactivity incorporated into the phospholipids was similar to that observed after 24-h exposure. However, differences in the phospholipid labeling profile were observed between the 24-h and the 4-d exposure. Data in Table 1 summarize the changes by expressing the ratio between the two data points. With the exception of PC, which did not change as a function of feeding time, substantial deviations from a ratio of 1.0 are evident for most of the lipids. For example, a ratio of less than 1 for PI indicated that the proportion of radioactivity in this phospholipid decreased during 4 d, and ratios greater than 1 for PS, PC_r and lysophospholipids indicate that the proportion of radioactivity of these phospholipids increased during 4 d of feeding. Interestingly, lysophospholipids tended to accumulate more in *norpA* than in wild type irrespective of lighting conditions. In this experiment, only 10 μCi/vial of ^{32}P was given, as it was expected that the 4 times longer feeding would balance the 5 times lower dose. However, incorporation was about 1/4, suggesting that most of the ^{32}P incorporation into phospholipids occurred during the first 24 h. On this basis, we conclude that this experiment simulates the pulse-chase type of study.

To further elaborate on these results, a time course experiment was carried out in which flies were analyzed at 3, 7, 16, 22 and 34 h after ^{32}P feeding. The data in Figure 4 show the distribution of radioactivity among the lipids for wild type (Fig. 4a) and *norpA* (Fig. 4b). In general, the phospholipid labeling patterns in *norpA* and wild type do not differ appreciably, but substantial changes in labeling pattern were observed with respect to time of ^{32}P feeding. For example, there is a time-dependent decrease

TABLE 1

Ratio of Chronic[a] over Acute[b] Incorporations into Selected Phospholipids of *Drosophila* Heads

Lipid[c]	*norpA* light	*norpA* dark	Wild light	Wild dark
PI	0.32575	0.40187	0.35354	0.38940
PS	2.10550	2.37810	3.15360	2.48900
PC	1.19600	1.07600	0.94984	0.92615
PCr	1.47800	1.60700	1.65740	2.25100
Sum LP	4.39600	4.03800	2.84500	3.36500
Sum PIP_2, PIP	1.27600	1.97200	2.20400	1.40000
PA	1.40000	0.89375	0.76000	0.92667

[a] Four-day feeding.

[b] One-day feeding.

[c] PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; LP, lysophospholipids; PIP_2, PI 4,5-bisphosphate; PIP, PI 4-phosphate; PA, phosphatidic acid; *norpA, no* receptor potential.

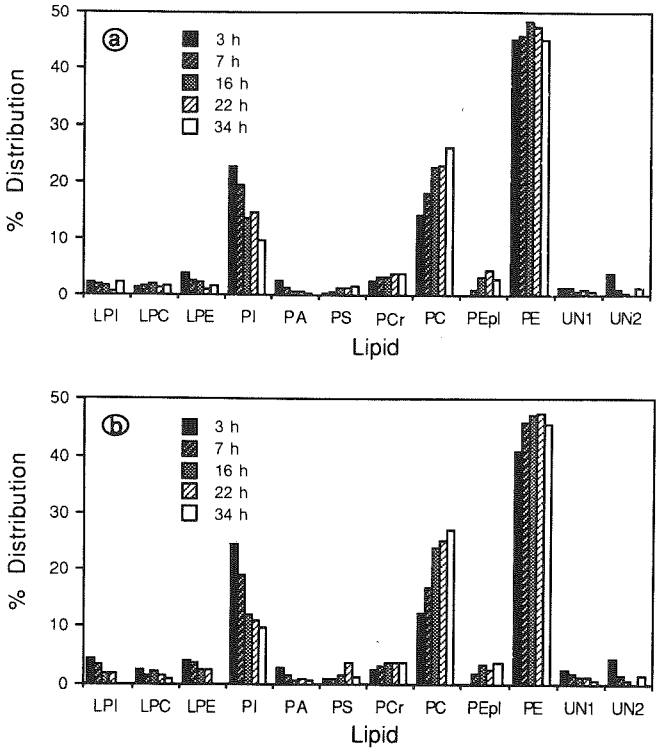

FIG. 4. Distribution of the radioactivity of phospholipids with respect to the duration of ^{32}P exposure: (a) for wild type; (b) for *norpA*. Abbreviations as in Figure 1.

in labeled PI, PA and lysophospholipids, and an increase in labeling of PC, PC_r and PS. Although label was highest in PE, the proportion of label associated with PE did not change greatly with time.

A study was performed to compare the phospholipid labeling pattern in aged *vs.* newly emerged flies. Although no profound changes were observed in the major phospholipids, aging may have increased the labeling of PIP and PIP_2 in wild type, but not in *norpA*, and decreased the labeling of LPC and LPI in all types (Fig. 5).

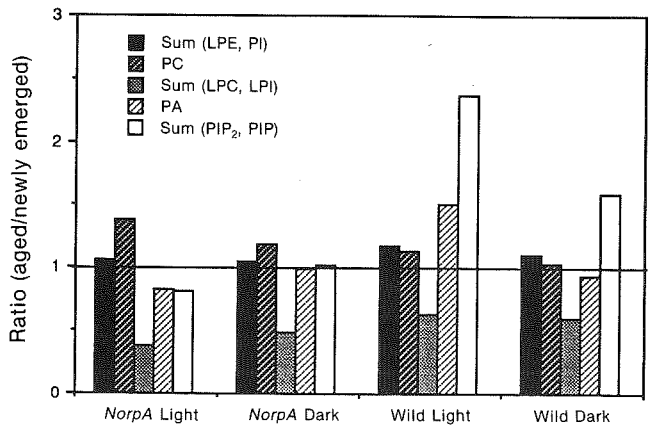

FIG. 5. Ratio of some representative phospholipids for flies aged one week compared with newly emerged flies. The ratio represents the percent distribution of lipids in the aged *vs.* newly emerged flies. Two plates each are pooled from one representative experiment for aged *vs.* newly emerged flies. Abbreviations as in Figure 1.

In pilot experiments, various time intervals were used for dissecting and sorting the heads, and we observed drastic differences in the labeling of lysophospholipids. For all of the data reported above, heads were more rapidly isolated by freezing in liquid nitrogen. When sample preparation was deliberately delayed, apparent lysophospholipid labeling was increased. These results seem to point at the presence of phospholipases in the head which would be responsible for the hydrolysis of the phospholipids.

Using the same feeding paradigm, *i.e.*, 24-h feeding of ^{32}P, labeling of major phospholipids (PA, PS, PI, LPE, PC and PE) was compared for the following groups: (i) wild type controls *vs. eya* (Fig. 6a); (ii) wild type controls *vs. ora* (aged, see methods) (Fig. 6b); (iii) β-carotene supplemented Sang's medium *vs.* Sang's medium which is deficient in carotenoid (Fig. 6c); and (iv) regular food (high carotenoid) controls with Sang's (high carotenoid) flies (Fig. 6d). This last comparison was important because the two diets differed in fatty acid composition (based on gas chromatography (GC) analysis, regular food has 16:0, 74.37%; 18:0, 13.61%; 18:1, 10.06%; and 18:2, 1.96%; whereas Sang's has 16:0, 61.13%; 18:0, 20.49%; 18.1, 18.38%; and 18:2, 0%). The data show that, despite the subtle differences that exist, the overall profiles for ^{32}P incorporation remain largely similar between the various groups.

DISCUSSION

Agonist stimulation of phosphoinositide hydrolysis by PLC is known to give rise to two second messengers, IP_3 and diacylglycerol (DG). The importance of IP_3 on Ca^{++} homeostasis (25) now explains the long realized importance of Ca^{++} on invertebrate photoreceptor function in transduction and/or adaptation. DG activates protein kinase C (PKC), two of which (one specific to the eye) have been cloned in *Drosophila* (26). DG can be converted to PA by DG kinase, an enzyme of interest in visual transduction as it is missing in the *rdgA* (*r*etinal *d*egeneration) mutant (27). In this study, PA is consistently labeled after exposure of flies to ^{32}P, suggestive of its role as an intermediate for phospholipid biosynthesis.

PLC has been shown to play a role in photoreceptor function in *Drosophila* (5). The *norpA* mutant is interesting because it has rhodopsin but lacks the receptor potential (21). It was proposed that this mutant is like dominantly inherited night blindness in humans and the *rd* (*r*etinal *d*ystrophic) chicken (for additional references, see ref. 20). *NorpA* loses rhodopsin as a function of age (3,28). It accumulates zipper-like membranes, suggested as a form of lipid storage abnormality (29). One of the original goals of this line of research was to design experiments to examine the phospholipids in this mutant and to understand how accumulation of lipids may occur during aging. As the data accumulated, it became obvious that it was also necessary to establish a method to label the membrane phospholipids and to present baseline data.

Our experiments revealed for the first time a novel method to label the phospholipids in *Drosophila* heads. By mixing $^{32}P_i$ with the food, effective labeling of the phospholipids in the heads can be achieved within a short time. Examination of the labeling pattern revealed high percentage of label distributed in PE (45%), followed by PC and PI. The ability to incorporate a large amount of

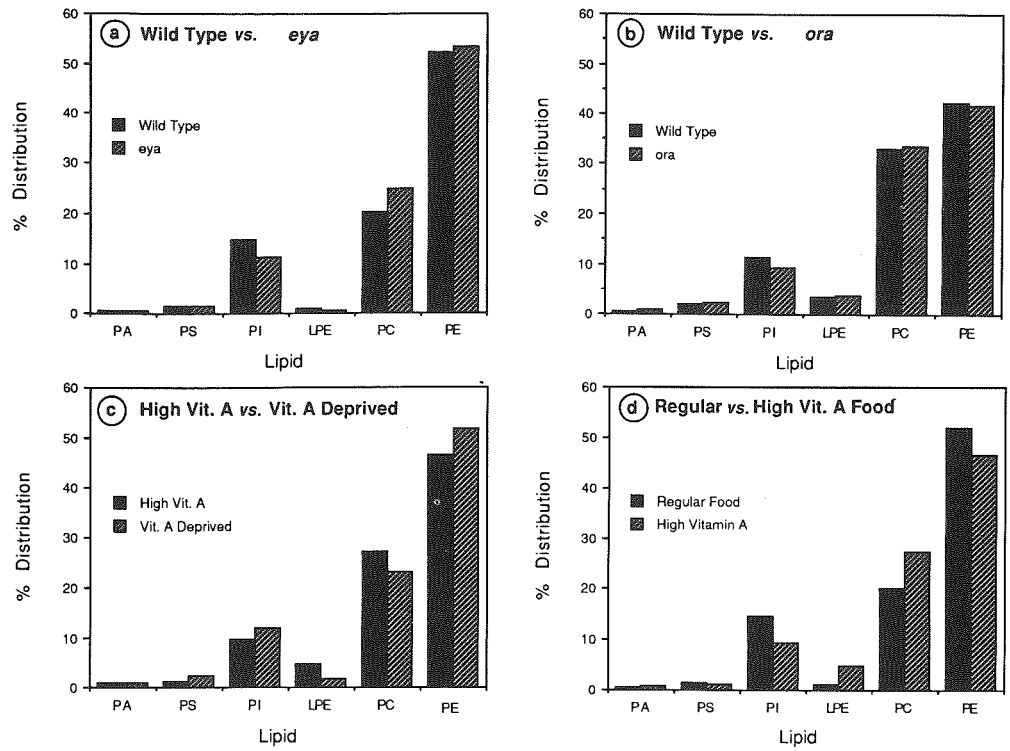

FIG. 6. The percentages of ^{32}P radioactivity in the major phospholipids (PA, PS, PI, LPE, PC and PE normalized to sum to 100%) from the heads of flies upon separation in two solvent systems. Fifty μCi of $^{32}P_i$ per vial was fed and 45 heads of deprived and 45 of the wild type were used. The sample was divided into three aliquots and spotted onto separate plates. The data from these plates were averaged. (a) Wild type *vs. eya.* All flies were newly emerged. (b) Wild type *vs. ora.* All flies were aged 11 d. (c) High β-carotene *vs.* β-carotene deprived flies. Heads were from flies of high β-carotene *vs.* deprived flies reared on Sang's medium. All flies were newly emerged. (d) Regular food *vs.* high Vitamin A food. The regular food control is the same as the *eya* control (Fig. 6a) while the Sang's is the high β-carotene for the vitamin A experiment (Fig. 6c). Abbreviations as in Figure 1. *Eya, eye*s *a*bsent; *ora, o*uter *r*habdomeres *a*bsent.

label into PE in the fly heads is interesting because PC is the predominant lipid in most mammalian tissues. The time course study revealed interesting differences in metabolism between PE *vs.* PI and PC, suggesting a role for PE in the structural make-up of the membrane. Unlike mammalian tissues, phospholipids in *Drosophila* heads are low in sphingomyelins and plasmalogens although the presence of various types of lysophospholipids is apparent. We were disappointed, though not surprised, that only trace amounts of label were incorporated into poly-PI, limiting the usefulness of our labeling procedure for further studies of the metabolism of inositol phospholipids.

Because $^{32}P_i$ is taken up by the flies as food, it is not possible to specifically address the labeling of phospholipids in the retina. In contrast, intracerebral injection of $^{32}P_i$ into mammalian brain can yield effective labeling of the poly-PI (14). Our inability to isolate retina from head, together with the fact that only trace amount of label is in poly-PI, may explain why we did not observe a difference in phospholipid labeling pattern between *norpA* and wild type, although it has clearly been established that the *norpA* mutant lacks PLC (10,11). Also, the *norpA* gene codes for PLC (12), which acts on the poly-PI as well as on PI (30). In the crab, an inositol trisphosphatase terminates the action of IP_3 in mediating visual transduction (31). In bovine brain, there are at least five different types of phospholipase C which are specific to inositol phospholipids (32).

We observed a difference in the overall labeling between *norpA* and wild type, likely attributable to a visually-mediated difference in the feeding behavior. Because *norpA* lacks the photoreceptor potential, these flies did not show a light-mediated increase in feeding. Another interesting observation is the comparison of phospholipid labeling between aged and newly emerged *norpA* and wild type flies. In both dark and light conditions, the aged wild type showed a ratio of greater than 1 for the labeling of poly-PI, whereas the ratios for *norpA* were not changed. The inability to show an age difference in the metabolism of poly-PI in *norpA* may reflect the defective metabolism of poly-PI turnover.

An interesting observation from this study is the presence of a significant amount of lysophospholipids in the heads, suggesting the presence of endogenous phospholipase A_2 (PLA_2) activity. In pilot experiments in which fly heads were not quickly broken off by freezing, we obtained evidence that varying amounts of lysophospholipids were released *post-mortem.* As the proportion of label of these lysophospholipids decreased in the chronic feeding (pulse chase) experiment (Table 1), it is reasonable to conclude that these compounds undergo metabolism and may form an integral part of the phospholipid

turnover cycle. Aging resulted in a decrease in the labeling of LPC and LPI, but there was no effect of mutant condition. We found that there was no effect of light on the appearance of lysophospholipids, though light seems to affect PLA_2 activity in vertebrate rods (33). In other systems, PLA_2 is known to serve as a direct source of arachidonate (34). However, analysis of the fatty acids of the phospholipids revealed no appreciable amount of arachidonic acid in *Drosophila* (data not shown). Zimmerman and Keys (35) characterized an enzymatic system responsible for the removal of LPC and suggested that the lysophospholipids generated by PLA_2 may be involved in the early stages of light-induced damage (35,36). In other studies, PLA_2 was shown to participate in "retailoring" of fatty acids in the 2-position (37). Thus, oxidized polyunsaturated fatty acids can be eliminated from the system through this mechanism. The lysophospholipid-acylCoA acyltransferases are important for recycling polyunsaturated fatty acids to the *sn*-2 position (38).

A comparison of the phospholipid labeling patterns was made among mutants including *norpA, ora* and *eya.* This type of "genetic dissection" is also underway in mouse (*rd* mutant) (39) and dog (*p*rogressive *r*od-cone *d*egeneration mutant) (40,41). We also dissected the system with carotenoid deprivation which selectively eliminates rhodopsin (42). This study suggests that the "annular lipids" are important for rhodopsin function (43).

Our results are consistent with the notion that $^{32}P_i$ is incorporated into phospholipids quickly and that some lipids do turnover more rapidly than others. Historically, in other systems, the fast turnover in membrane lipids heralded the discovery of lipid involvement in messenger systems. Our results provide the initial characterization of membrane phospholipids of *Drosophila* heads in wild type and vision mutants. This information will serve as an important base for understanding the mechanism of phototransduction. Future directions are likely to center around the functional role of lipids in visual receptors.

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