Vol. 111, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1983 Pages 567-573

DEFECTIVE PHOSPHOLIPID METABOLISM IN THE RETINULAR CELL MEMBRANE OF norpA (NO RECEPTOR POTENTIAL) VISUAL TRANSDUCTION MUTANTS OF DROSOPHILA

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Received January 4, 1983

SUMMARY : The phosphorylation of photoreceptor phospholipids in the three alleles of *Drosophila* visual mutants (*norpA* : no receptor potential A gene) was studied. In the normal strain, the γ-^{32}P of ATP was transferred mainly to phosphatidic acid (PA) and diphosphoinositide (DPI), while, in the mutants, we found that the phosphorylation of PA was drastically reduced, but that of DPI was not. The radioactivity incorporation into PA closely parallels with the degree of the mutant genes' expressivity among the three alleles of *norpA* tested. Therefore, the abnormality found in the phosphorylation of diglycerol to PA may be closely related to the primary mutant defect in the phototransduction mechanism.

A number of Drosophila mutations have been isolated which cause defects in the adult vision. Many of them were shown to have their primary site of gene action in the compound eyes by means of the genetic mosaic technique (1). Among them, norpA (no receptor potential A) is one of the most interesting genes, whose mutations cause reduction or absence of the receptor potentials. Since both gross morphology and rhodopsin content of the rhabdomere were found to be normal in the mutants (2), it is suggested that they harbor a defect in the transduction mechanism between light reception and changes in ion permeabilities of the retinular cell membrane. Indeed, we could identify reduction of intramembrane particles in the mutant rhabdomere membrane by means of the freeze-fracture technique (3). We also found that the amount of the two major rhabdomeric proteins in these mutants are reduced without any detectable changes in their molecular weights nor isoelectric points (4).

Recently, phosphorylated proteins and a special class of phospholipids have been implicated in the regulation of membrane permeability of various

0006-291X/83 $1.50

type of cells (5,6,7). In the visual systems, rhodopsin was found to be phosphorylated after bleeching in vertebrate and invertebrate photoreceptors (8,9). Matsumoto et. al. (10) reported that light dependent phosphorylation of a special class of polypeptides in Drosophila eyes does not take place in a norpA mutant. In the study of squid retina (11), it was found that phosphorylation of phospholipids was much higher than that of proteins. Among the phospholipids, incorporation of ^{32}P into diphosphoinositide (DPI) and phosphatidic acid (PA) was found to be affected by light irradiation. Similar results were also obtained in octopus retina (12).

In this report, we demonstrate that the amount of PA in the compound eye of norpA mutants were abnormal; degree of the change being closely related with the severity of the syndrome among the three alleles tested. A possibility of the importance of PA in the photoreception is discussed.

MATERIALS AND METHODS

Materials : Canton-S strain of Drosophila melanogaster was used as a standard, normal strain, from which the mutations were induced with an alkylating agent ethyl methanesulfonate. The independently isolated alleles of norpA genes used were $norpA^{EE5}$, $norpA^{SB37}$ and $norpA^{JM11}$ in the order of severity of the genetic disorders. Hereafter, they are designated as EE5, SB37 and JM11, respectively. Flies were collected within 1 day after eclosion. They were kept on fresh cornmeal-agar-yeast food until they become 4 to 7 days old. Twenty-four hours before experiments, they were transferred to complete darkness. Immediately before the experiment, flies were frozen with dryice and were then decapitated manually on a dryice plate under dim red light. Heads were collected and homogenized by glass homogenizer in the reaction mixture containing 100 mM KCl, 10 mM NaCl, 10 mM $MgCl_2$, 1 mM EGTA and 20 mM phosphate buffer (pH 6.8). γ-^{32}P ATP(2,000 Ci/mmol) was purchased from New England Nuclear Co.

Quantitative analysis of phospholipid : Phospholipids were extracted and separated by the method of Jolles et.al. (13) with the following modification. Lipids were extracted from perchloric acid (PCA) precipitates with 1 ml of ice cold mixture of chloroform/methanol/12N HCl (200:100:0.75, v/v) and a biphasic system was formed by adding 0.2 ml of 1 N HCl. The upper phase was removed and the lower phase was washed three times with its synthetic upper phase. Extracted lipids were separated by thin-layer chromatography (TLC) on a silica gel plate (Art 5721, Merck). The plate was developed in the first dimension with chloroform/methanol/28% NH_4OH (65:35:5, v/v) and in the second dimension with chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, v/v). The lipids were detected by iodine vapor and spots were scraped from the plate and digested by the method of Farese et. al. (14) with the following modifications. Scraped spots were heated for 1.5 hour at 200°C with 0.7 ml of 14% sulfuric acid and 12% PCA (2:1,v/v) and phosphorus was measured by the micromethod of Bartlet (15). Protein concentration was determined by the method of Lowry et. al. (16) using bovine serum albumin as a standard.

Phosphorylation assay : About 100 heads were homogenized in 0.5 ml of the reaction mixture described above. The volume of the sample was adjusted to make final concentration as 20 heads/ml. Phosphorylation was started by adding γ-^{32}P ATP (10 μCi/ml). After incubation for two minutes at 24°C, the aliquot was shaken with an equal amount of ice cold PCA in order to stop the reaction. Lipid was extracted by the same procedure described above. Since radioactivity was found to be concentrated in acidic phospholipids, we analysed it by one dimensional TLC, developed with the mixture of chloroform/acetone/methanol/acetic acid/water (40:17:15:12:8, v/v). The TLC plates (Art. 5631, Merck) were impregnated in 1% potassium-oxalate solution before use. The lipids were detected with iodine vapor, and ^{32}P incorporations were detected by autoradiography, and their radioactivities were counted by scintillation counter.

RESULTS

Phospholipid phosphorylation of whole head homogenate :

We first present evidence to indicate that, under the experimental conditions used here, most of the ^{32}P of ATP incorporated into the phospholipids in the head homogenate can be explained by the uptake into retinular (photoreceptor) cell components by using a mutant called rdgA^{KO14} (receptor degeneration A gene). This mutation has been shown to cause post-eclosional degeneration of peripheral retinular cells and their rhabdomere without affecting gross morphology of other cell types. We made experiments on rdgA^{KO14} head homogenate and could show that ^{32}P incorporations into phosphatidic acid (PA), diphosphoinositide (DPI) and triphosphoinositide (TPI) was reduced to very low level (Fig.1b). Similar results were also obtained by examining *sine oculis* mutant which lack entire compound eyes and a part of the associated optic ganglia (Fig.1c). These data support our notion that we can essentially measure ^{32}P uptake into retinular cell phospholipids by simply measuring total incorporation into head homogenate.

Defects in retinular cell phospholipid metabolism among norpA mutants :

Three alleles of norpA mutants, JM11, SB37 and EE5 were compared with the normal strain. As is shown in Fig.1 and Fig.2, γ-^{32}P of ATP was transferred to PA, DPI and TPI in the normal strain. However, in EE5, ^{32}P incorporation into PA was suppressed to only 10% of that in normal. The approximate ratio of the radioactivity in PA was normal : JM11 : SB37 : EE5 = 1: 0.5: 0.5: 0.1 (Fig.2). This agreed well with the order of the mutant genes' expressivity

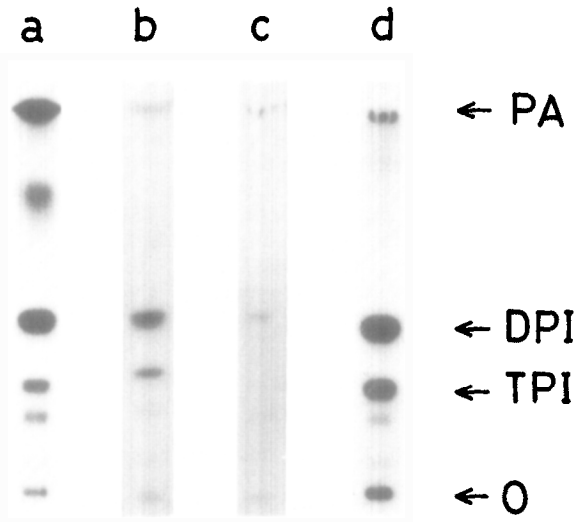

Fig. 1 Autoradiographs after thin layer chromatography (TLC) of phospholipids extracted from the head homogenates, a: normal strain ; b: rdgA^{K014} (mutant without peripheral rhabdomere); c: sine oculis (mutant without eyes); d: norpA^{EE5} (mutant without receptor potentials).

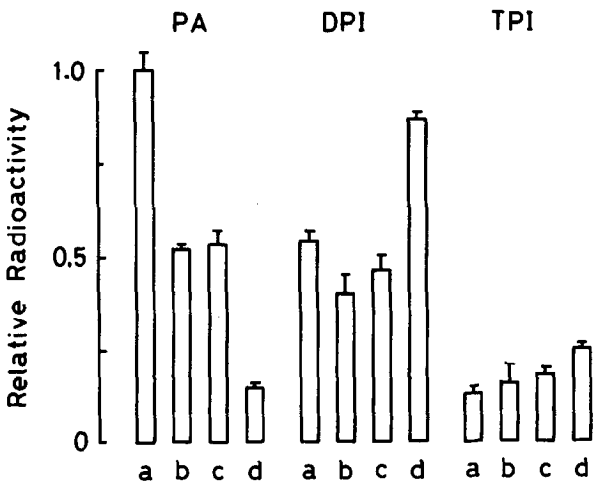

Fig. 2 Radioactivities of ^{32}P-incorporated phospholipids scraped from the plate after TLC. a: normal strain (N=25); b: norpA^{JM11} (N=5); c: norpA^{SB37} (N=5); d: norpA^{EE5} (N=12). Incubation (2 min) was carried out as described in Materials and Methods. Bars in the figure indicate standard deviations. PA: phosphatidic acid, DPI: diphosphoinositide, TPI: triphosphoinositide, O: origin.

Table 1 Percent distribution of phospholipids in whole heads of Drosophila

	% Pi among total Pi of phospholipids				
	P E[a]	P C[a]	P I[a]	P S[a]	P A[b]
Normal	58.5 ± 4.2	34.7 ± 3.3	4.1 ± 1.1	2.7 ± 0.6	+
JM11	60.5 ± 4.5	34.2 ± 4.2	2.7 ± 0.8	2.6 ± 0.7	-
SB37	61.5 ± 3.4	33.1 ± 4.3	3.6 ± 0.6	1.8 ± 0.4	-
EE5	61.8 ± 5.3	33.6 ± 5.6	2.6 ± 0.3	2.0 ± 0.4	-

a. Average of 5 determinations, in which phospholipids were extracted from 200 heads. Each value represents mean% of Pi among the total phospholipids ± standard deviation.
b. The content of PA was below the detectability of chemical analysis. Therefore, its presence was semiquantitatively determined by iodine vapor staining.

Abbreviations PE: phosphatidylethanolamine, PC: phosphatidylcholine, PI: phosphatidylinositol, PS: phosphatidylserine, PA: phosphatidic acid

judged by the size of the receptor potentials. The radioactivity taken up into TPI, on the other hand, was in the reverse order. DPI did not show such a correlation with the mutant syndrome.

Quantitative analysis of phospholipids :

To determine whether such abnormality is reflected in the phospholipid composition, we collected 200 fly heads (1.7 mg protein) for chemical analysis. Total phospholipid phosphorus in the head homogenates of the three mutant alleles are not different from that of normal flies: the average being 19.1 ± 1.2 μg phosphorus/mg protein. As is shown in Table 1, relative composition of the phospholipids was also normal in the three mutants. On the other hand, the content of PA is too low for the chemical analysis. It is barely detectable by the more sensitive iodine vapor technique in the normal strain, but not in the mutant heads. To determine PA content more accurately, 1,000 fly heads (8.5 mg protein) were analyzed. Phosphorus of PA in normal homogenate was found to be about 0.3 μg, but that in the three mutant alleles was still below the detectability of the technique.

DISCUSSION

The molecular mechanisms of phototransduction is still not understood. For such an analysis, genetic dissection of the process will be a promising

approach. The X-linked norpA mutations of Drosophila were chosen for this study, since it causes deficits in the transduction processes. Although little is known about the primary product of the gene, it is considered to be a protein, because some of the alleles are temperature-sensitive. However, molecular weight and isoelectric point of the two major proteins in the rhabdomere are normal among the six alleles examined, though their amount in the mutants are significantly reduced (3). We could also demonstrate that number of the intramembrane particles of the rhabdomere were remarkably reduced in some of the alleles including in EE5 (4).

In this study, we proved that incorporation of radioactivity from γ-^{32}P of ATP into PA of the mutants' photoreceptor membrane was decreased. The degree of this anomaly is in parallel with the size of the receptor potentials in the three mutant alleles which were independently isolated after chemical mutagenesis. These data sugget a close correlation between the defects in the phosphorylation and those in the transduction and receptor potential mechanisms. Since PA is mainly made by phosphorylation of diglycerides (DG) in the presence of DG-kinase and ATP, the abnormality indicates either (i) a reduced amount of DG-kinase, (ii) partial inhibition of DG-kinase or (iii) decrease in DG content in the mutants.

Recently there are many reports suggesting a relationship between PA and membrane permeabilities, especially for calcium ions in various types of cells like rat parotid (17) and platelet (18). It is, therefore, possible that PA itself constitutes a part of ionic channels involved, or that independent channel molecules do not function properly in the absence of sufficient amount of PA in the membrane.

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