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A Comparative Study of Adrenal Phospholipid Fractions in Avian Species

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Eight different adrenal phospholipid fractions, viz. phosphatidic acid, cardiolipin, phosphatidyl ethanolamine, phosphatidyl glycerol, lecithin, sphingomyelin, lysolecithin and phosphatidyl inositol were identified in the nine avian species studied by us showing no significant phylogenetic trend or dependence on the relative concentrations of epinephrine and norepinephrine. Medulla contained high amount of phospholipids. The major fractions phosphatidyl ethanolamine, lecithin and sphingomyelin are suggested to play a significant role in the secretory process in the chromaffin cells.

Key Words: **Phospholipid, Adrenal, Bird**

Introduction

Considerable work has been done on the biochemical composition of the catecholamine-storing granules of adrenal medulla. These possess a biochemical complexity not reflected by their deceptively simple morphology (Winkler 1976). The soluble proteins except dopamine β-monooxygenase have often been postulated to exist as complexes with the non-protein soluble components, which include catecholamines, nucleotides, lipids, mucopolysaccharides and ascorbate. Several authors maintain that all of the soluble proteins occur in complex with lipids as low or high density lipoproteins (Koeing 1974). Sphingomyelin, lecithin and cholesterol which appear to be the primary lipid components of the lipoprotein complex, account for about 9, 19 and 25% of total granule lipid respectively (Winkler 1976). It has already been reported that the characteristic feature of the mammalian chromaffin granules is a high content of lysolecithin (Blaschko et al. 1967, Da prada et al. 1972, and Koenig 1974) and cardiolipin (Blaschko et al. 1967) but low level of phosphatidylinositol (Da Prada et al. 1972). Although the study of different fractions of adrenal phospholipid has been fairly well documented in mammals such has not been systematically done in birds so far. Ghosh

Table 1 *Adrenal phospholipid profile of nine avian species*

Species orders and families	Total phospholipid (mg/g tissue)	Percentage of individual phospholipid content								ANOVA
		Phosphatidic acid (PA)	Cardiolipin (C)	Phosphatidyl ethanolamine (PE)	Phosphatidyl glycerol (PG)	Lecithin (L)	Sphingomyelin (S)	Lysolecithin (LL)	Phosphatidylinositol (PI)	
1. *Ardeola grayii* (O : Ciconiformes, F : Ardeidae)	24.15±1.86*	4.8	1.8	25.0	2.0	24.5	34.2	4.7	3.0	$p < 0.01^a$
2. *Anas platyrhynchos* (O : Anseriformes, F : Anatidae	11.08±0.98	5.9	1.8	37.4	2.9	29.2	19.6	2.2	1.0	$p < 0.01^a$
3. *Dinopium benghalense* (O : Piciformes, F : Picidae)	20.62±2.08	4.6	2.0	27.0	1.8	22.5	34.4	4.5	3.2	$p < 0.01^a$
4. *Columba livia* (O : Columbiformes, F : Columbidae)	52.16±4.32	7.2	2.5	31.4	3.2	28.6	22.2	3.4	1.5	$p < 0.01^a$
5. *Athene brama* (O : Strigiformes, F : Strigidae)	23.36±4.79	5.6	2.2	30.4	2.2	28.1	25.7	3.8	2.0	$p < 0.01^a$
6. *Psittacula krameri* (O : Psittaciformes, F : Psittacidae)	18.62±4.20	3.4	1.4	41.2	4.5	34.2	13.4	1.2	0.7	$p < 0.01^a$
7. *Corvus splendens* (O : Passeriformes, F : Corvidae)	10.23±1.11	6.7	3.1	30.0	0.6	31.4	25.2	2.2	0.8	$p < 0.01^a$
8. *Pycnonotus cafer* (O: Passeriformes, F : Pycnonotidae)	50.04±11.87	6.9	3.2	25.8	1.8	32.6	24.3	4.2	1.2	$p < 0.01^a$
9. *Acridotheres tristis* (O : Passeriformes, F : Sturnidae)	31.42 ± 2.62	6.5	3.0	30.6	3.6	29.1	20.5	4.8	1.9	$p < 0.01^a$
ANOVA	$p < 0.01^a$	NS	NS	NS	NS	NS	NS	NS		$p < 0.01a$

*Mean ± Standard Error. *ANOVA*: Analysis of variance; a, Significant at 1% level; *NS* : Not Significant

and his collaborators (Ghosh 1962) demonstrated that acid phosphatase, metachromatic materials and plasmalogen were the basic cytochemical unit of all the twenty-one avian species studied by them. Further, it has been reported that phospholipids are involved in the secretion of proteins by glandular tissue and also in the secretion of epinephrine from the adrenal medulla of guineapig (Hokin & Hokin 1958). In view of this, it seems worthwhile to make a detailed comparative biochemical

study of the phospholipid profile of the adrenal of birds belonging to different phylogeny.

Materials and Methods

Nine young-adult avian species of different phylogenetic order, mostly including both sexes were obtained from a local bird dealer (6 birds in each case, table 1). They were kept in the laboratory conditions for at least three days for acclimatization with *ad libitum* food and water. Then the birds were killed by cervical dislocation. From each bird, the adrenal glands were quickly dissected out and processed for the estimation of phospholipid. For the qualitative and quantitative measurements of the phospholipid fractions, lipids were extracted with chloroform and methanol (2:1 v/v) from fresh adrenal tissue and purified (Folch et al. 1957). The extracting solvent was reduced and the reduced solvent was further extracted with chloroform. Total adrenal phospholipids were estimated. The two-dimensional thin layer chromatography for the identification of the phospholipid fractions was then carried out by the method of Batabyal et al. (1984). The removal of the spot and elution of phospholipids were done by the method of Skipski et al. (1964). Concentrations of individual phospholipid fraction were estimated by the standard method. Standard phospholipids were obtained from M/S Sigma Chemicals Co., USA. The biochemical data of total adrenal phospholipid and individual fractions of all the birds and different

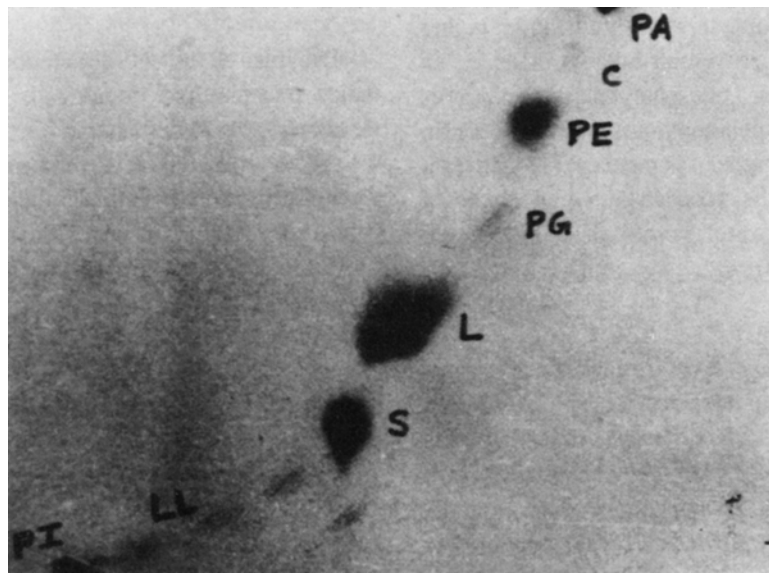

Figure 1 Normal chromatogram of eight adrenal phospholipids of pigeon — **1**, Phosphatidic acid (PA), **2**, Cardiolipin (C), **3**, Phosphatidyl ethanolamine (PE), **4**, P-glycerol (PG), **5**, P-choline (lecithin) (L), **6**, Sphingomyelin (S), **7**, P-Inositol (PI) and **8**, Lysolecithin (LL).

phospholipid fractions in individual birds were analysed statistically by analysis of variance (ANOVA) (Snedecor & Cochran 1967).

Results

The biochemical computations are summarised in table 1. Eight different phospholipid fractions viz. phosphatidic acid, cardiolipin, phosphatidyl ethanolamine, phosphatidyl glycerol, lecithin, sphingomyelin, lysolecithin and phosphatidyl inositol were clearly resolved and chromatographed in all the birds against known standards (figure 1). The molar contents of the individual phospholipid fractions were expressed as percentages of total phospholipid. Adrenal phospholipid content was significantly higher in pigeon and redvented bulbul (table 1). In all the species, phosphatidyl ethanolamine, lecithin and sphingomyelin were found in significantly higher percentage. Significant variations in the percentage composition of phosphatidyl inositol were noticed in different species studied.

Discussion

Our findings have revealed that phospholipids were present in considerable amount in the adrenal gland of different avian species studied. All the earlier cytochemical as well as biochemical investigations done on the adrenal phospholipid in higher vertebrates (birds and mammals) showed predominance of the phospholipid in the medulla (Petit et al. 1995). Therefore, the high amount of avian adrenal phospholipid estimated biochemically in the present study mostly accounted for the medullary phospholipid content. Again an overall similarity in the appearance of all the eight different fractions chromatographed was noticed in all the nine phylogenetically different avian species. But quantitatively, percentage composition of these individual fractions except phosphatidyl inositol, did not differ significantly from species to species. An interesting question therefore may arise from these findings here — whether this difference in the molar percentage of phosphatidyl inositol was due to the difference in phylogenetic status or due to various concentrations of epinephrine and norepinephrine in them? A perusal of table 1 indicates that this restricted data on the adrenal phospholipid profile in birds neither exhibit any significant phylogenetic trend nor the relative concentrations of epinephrine and norepinephrine (Ghosh & Ghosh 1962) reflected any relationship with this fraction.

Dreyfus et al. (1978) reported that the major phospholipid compounds in mammals were phosphatidylethanolamine and lecithin whereas minor phospholipids include phosphatidylserine, phosphatidylinositol, lysophosphatidylethanolamine, choline plasmalogen and phosphatidic acid and suggested that the relatively high amount of phospholipid components of the chromaffin granule membranes might be necessary for the fusion of the granules with the plasma membrane of the chromaffin cell. In recent years, several other investigators have opined a similar role of the phospholipids in the secretory process of the chromaffin granules (Kuijpers et al. 1992). Therefore it can be assumed from the present study that the major phospholipid fractions (phosphatidylethanolamine, lecithin and sphingomyelin) found in all the nine avian species investigated, might play a significant role in the secretory process in the chromaffin cells. Further investigation, in future, to see the involvement of the minor phospholipids reported here, in the secretory process in

the chromaffin cells, may bring forth useful informations.

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References

Batabyal S K, Deb S and Bose S N 1984 A quick method of two dimensional thin layer chromatographic seperation of human serum phospholipids and their identification; *J. Indian Chem. Soc.* **LXI** 176-178

Blaschko H, Firemark H, Smith A D and Winkler H 1967 Lipids of the adrenal medulla : lysolecithin, a characteristic constituent of chromaffin granules; *Biochem. J.* **104** 545-549

Da Prada M, Pletscher A and Tranzer J P 1972 Lipid composition of membranes of amine-storing organelles; *Biochem. J.* **127** 681-683

Dreyfus H, Pescheloche M, Harth S, Mandel P and Aunis D 1978 Lipids in bovine adrenal-medullary chromaffin granules : structure, location and accessibility in the membrane of gangliosides and lactosylceramide sialytransferase activity; *Biochem. Soc. Trans.* **6** 312-314

Folch J, Lees M and Stanley G H S 1957 A simple method for the isolation and purification of total lipids from tissues; *J. Biol. Chem.* **226** 407-409

Ghosh I and Ghosh A 1962 A comparative cytochemical study on the avian suprarenal medulla; *Proc. Zool. Soc.* **15** 135-145

Ghosh A 1962 A comparative study of the histochemistry of avian adrenals; *Gen. Comp. Endocrinol.* Suppl. **1** 75-80

Hokin M R and Hokin L E 1958 Phospholipids and adrenaline secretion in guineapig adrenal medulla; *J. Biol. Chem.* **233** 814-817

Koenig H 1974 The soluble acidic lipoproteins (SALPS) of storage granules : Matrix constituents which may bind stored molecules; in *Advances in Cytopharmacology* Vol. 2, 273-301. eds. B Ceccarelli, F Clementi and J Meldolesi (New York : Raven Press).

Kuijpers G A, Lee G and Pollard H B 1992 Imunolocalization of synexin (annexin VII) in adrenal chromaffin granules and chromaffin cells : Evidence for a dynamic role in the secretory process; *Cell Tissue Res.* **269** 323-330

Petit K, De-Block J and De Potter W 1995 Isolation and characterization of a cytosolic phospholipase A2 from bovine adrenal medulla, *J. Neurochem.* **64** 139-146

Skipski V P, Peterson R F and Barclay M 1964 Quantitative analysis of phospholipids by thin layer chromatography; *Biochem. J.* **90** 374

Snedecor G W and Cochran W G 1967 *Statistical Methods* 6th ed. (Ames : Iowa State Univ. Press)

Winkler H 1976 The composition of adrenal chromaffin granules : an assessment of controversial results; *Neuroscience* **1** 65-80