Lipid Composition of Membranes of Amine-Storage Organelles

By M. DA PRADA, A. PLETSCHER and J. P. TRANZER

Research Department, F. Hoffmann-La Roche and Co. Ltd., Basle, Switzerland

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1. The lipid composition of the membranes from isolated 5-hydroxytryptamine-storage organelles of blood platelets of rabbits and of those from chromaffin granules of bovine adrenal medulla was compared. 2. In contrast with the membranes of the chromaffin granules, those of the 5-hydroxytryptamine organelles did not contain lysophosphatidylcholine (lysolecithin). 3. Both the cholesterol/phospholipid ratio and the relative proportions of phosphatidylethanolamine (cephalin), phosphatidylinositol and phosphatidylserine were about the same in both membranes, whereas phosphatidylcholine (lecithin) and sphingomyelin showed somewhat higher values in the membranes of the 5-hydroxytryptamine organelles. 4. In conclusion, the release of 5-hydroxytryptamine from blood platelets is probably not correlated with the presence of lysophosphatidylcholine in the membranes of the storage organelles and may thus differ from the mechanism of catecholamine release in adrenal medulla.

The membranes of chromaffin granules of bovine adrenal medulla contain considerable amounts of lysophosphatidylcholine (lysolecithin) (Blaschko *et al.*, 1967; Winkler *et al.*, 1967; Smith, 1968; Winkler, 1971). This phospholipid is thought to be involved in the release of catecholamines by exocytosis of the granular contents. Exocytosis may be the consequence of a fusion of the granular with the cytoplasmatic membranes induced by lysophosphatidylcholine (De Robertis & Vaz Ferreira, 1957; Schneider *et al.*, 1967; Diner, 1967; Douglas, 1968).

Another type of amine-storage organelles, i.e. the 5-hydroxytryptamine organelles of blood platelets, has been isolated in a virtually pure form (Da Prada *et al.*, 1967). The function of the membrane of these organelles in the process of 5-hydroxytryptamine release is not known. It was therefore decided to compare the lipid composition of the membranes of the 5-hydroxytryptamine organelles of blood platelets with those of the adrenal chromaffin granules.

Materials and Methods

All chemicals used were of analytical reagent or the best commercially available grade. The phospholipids used as standards were purchased from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A. Diethyl *p*-nitrophenyl phosphate (E 600) was from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.

Isolation of organelles

The subcellular organelles storing 5-hydroxytryptamine or catecholamines were isolated by ultracentrifugation of homogenates of isolated rabbit platelets and bovine adrenal medulla respectively,

as previously described (Da Prada & Pletscher, 1968; Smith & Winkler, 1967). The organelles (from 500 ml of platelet-rich plasma and 0.5 g of adrenal medulla respectively), suspended in 4 ml of water, were disrupted by freezing and thawing (three times) and the membranes sedimented by centrifugation at 114483g (r_{av} 6.4 cm) for 15 min at 4°C. The sediment was washed twice with ice-cold water to remove amines, nucleotides and other soluble contaminants.

In some experiments, the adrenal medulla was homogenized in the presence of the phospholipase inhibitor diethyl *p*-nitrophenyl phosphate (50 mM). The inhibitor was emulsified in either 0.3 M-sucrose or in 0.3 M-sucrose containing 0.04% deoxycholic acid.

Extraction and assay

The identification and assay of the lipids were carried out by using the slightly modified procedures indicated by Rouser *et al.* (1970). Accordingly, the lipids were extracted from part of the membrane sediment with approx. 20 vol. of nitrogen-saturated chloroform-methanol (2:1, v/v) at 4°C. Another part was submitted to protein analysis (Lowry *et al.*, 1951).

The lipid extracts were washed with 0.05 M-KCl (Folch *et al.*, 1957) and submitted to one-dimensional two-step t.l.c. (Skipski *et al.*, 1965). The separation of the phospholipids was carried out by two-dimensional t.l.c. (Rouser *et al.*, 1970). For identification, the spots were developed with 2',7'-dichlorofluorescein (general lipid reagent) (Mangold, 1961), MnO₂ (cholesterol reagent) (Goswami & Frey, 1970), ammonium molybdate (for phosphorus) (Vaskovsky & Kostetsky, 1968), ninhydrin (for free amino groups)

or u.v. light (for nucleotides). For the quantitative assays, the spots were developed with iodine vapour and were scraped off and analysed for phosphorus content, which was multiplied by 25 to obtain the phospholipid values (Rouser *et al.*, 1966). Cholesterol was determined by a spectrophotofluorimetric method (Solow & Freeman, 1970) in the total lipid extract. The recoveries for phospholipids and cholesterol amounted to 95–100%.

Electron microscopy

The various sediments, consisting of isolated storage organelles or of their membranes, were fixed in a solution of glutaraldehyde (3%, v/v) and sodium-potassium phosphate buffer (0.1 M, pH 7.4, at 4°C for 2–4 h. Subsequently, a second fixation was performed for 1 h at 4°C with a solution of OsO₄ (2%, v/v) in the same phosphate buffer. The tissue blocks were dehydrated in ethanol and propylene oxide and embedded into Epon. The ultrathin sections [50 nm (500 Å)] were contrasted with uranyl acetate and lead citrate and examined with a Philips EM 300 electron microscope.

Results

Purity of membranes

The isolated 5-hydroxytryptamine organelles of rabbit platelets and the chromaffin granules of bovine adrenal medulla showed a high degree of purity as indicated by electron microscopy (Plate 1). Virtually no other subcellular organelles, such as mitochondria, microsomal fractions or α -granules (from platelets), were present. By hypo-osmotic shock of the isolated organelles pure membrane preparations were obtained (Plate 2).

Lipid composition of membranes

The ratio cholesterol/total phospholipids did not show a significant difference ($P < 0.05$) between the membranes of platelet organelles and chromaffin granules, but the amounts of cholesterol and phos-

pholipids (in mg/mg of protein) were somewhat higher in the platelet organelles (Table 1). In both types of membranes, the cholesterol fraction consisted of unesterified cholesterol and traces of esterified cholesterol, whereas the phospholipid fraction was composed of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol and phosphatidylserine. In addition, the membranes of the chromaffin granules contained a considerable amount of lysophosphatidylcholine, whereas this phospholipid was not present in membranes of 5-hydroxytryptamine organelles (Table 2). The relative amounts of phosphatidylcholine and sphingomyelin (related to the total phospholipids) were somewhat higher in the membranes of the 5-hydroxytryptamine organelles than in those of the chromaffin granules, whereas the other phospholipids showed similar values in both types of membranes (Table 2). No mono-, di- and tri-glycerides or contaminating nucleotides were found, but traces of free fatty acids and a non-identified phospholipid were detected.

Inhibition of phospholipase

Granular membranes of adrenal medulla, homogenized in the presence of the phospholipase inhibitor diethyl *p*-nitrophenyl phosphate, contained $13.0 \pm 0.4\%$ lysophosphatidylcholine (related to the total amount of phospholipids). Membranes not exposed to the inhibitor showed a somewhat higher content of lysophosphatidylcholine ($17.4 \pm 1.0\%$; see Table 2).

Discussion

The membranes of platelet organelles and chromaffin granules differ mainly with regard to their content of lysophosphatidylcholine: only the latter contains this phospholipid. The 5-hydroxytryptamine organelles of platelets thus resemble the sympathetic-nerve-trunk vesicles, since these have been shown to be rather poor in lysophosphatidylcholine (Lagercrantz, 1971). The physiological significance of the higher values of phosphatidylcholine and sphingomyelin in membranes of rabbit organelles compared with those of bovine chromaffin

Table 1. *Quantitative determination of total phospholipids and free cholesterol in membranes of 5-hydroxytryptamine organelles and chromaffin granules*

For experimental details see the Materials and Methods section. Values are expressed in mg/mg of protein and represent averages with S.E.M. of six to ten experiments.

	Chromaffin granules	5-Hydroxytryptamine organelles
Cholesterol (C)*	0.252 ± 0.033	0.153 ± 0.006
Phospholipids (P)*	1.293 ± 0.153	0.772 ± 0.028
Molar ratio C/P	0.395 ± 0.030	0.423 ± 0.007

* Differences between 5-hydroxytryptamine organelles and chromaffin granules are significant ($P < 0.01$).

EXPLANATION OF PLATE I

Electron micrograph of isolated 5-hydroxytryptamine organelles of rabbit platelets (a) and chromaffin granules of bovine adrenal medulla (b). Magnification $\times 18000$.

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EXPLANATION OF PLATE 2

Electron micrograph of isolated membranes of 5-hydroxytryptamine organelles (a) and adrenal chromaffin granules (b). Magnification $\times 18000$.

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Table 2. Content of the various phospholipids in the membranes of 5-hydroxytryptamine organelles and chromaffin granules

For experimental details see the Materials and Methods section. Values are given as percentages of the total phospholipid fraction (=100) and represent averages with S.E.M. of three to five experiments.

	Chromaffin granules	5-Hydroxytryptamine organelles
Phosphatidylcholine*	26.3 ± 0.7	33.8 ± 0.9
Phosphatidylethanolamine	34.8 ± 1.1	31.9 ± 1.1
Lysophosphatidylcholine*	17.4 ± 1.0	Nil
Sphingomyelin*	12.0 ± 0.6	17.1 ± 1.6
Phosphatidylinositol	1.5 ± 0.4	2.5 ± 0.8
Phosphatidylserine	8.9 ± 0.6	11.9 ± 1.2

* Differences between 5-hydroxytryptamine organelles and chromaffin granules are significant ($P < 0.01$).

granules remains to be investigated. It is noteworthy that blood plasma shows lower values of these phospholipids in rabbits than in cows (Rouser *et al.*, 1968).

The experiments with diethyl *p*-nitrophenyl phosphate indicate that formation of major quantities of lysophosphatidylcholine from phosphatidylcholine in the membranes of chromaffin granules during the isolation procedure is unlikely. In fact, when this potent inhibitor of phospholipase was added to the homogenization medium, the content of lysophosphatidylcholine in the granular membranes of the adrenal medulla did not markedly diminish. In contrast, the inhibitor induced a considerable decrease (by more than 90%) of the lysophosphatidylcholine in pancreatic microsomal fractions (Meldolesi *et al.*, 1971).

These findings indicate that the 5-hydroxytryptamine of the platelets can probably be released without the presence of lysophosphatidylcholine in the membrane of the 5-hydroxytryptamine organelles. According to preliminary results (unpublished work in this laboratory) the cytoplasmic membranes of the platelets are practically devoid of this phospholipid. Therefore the 5-hydroxytryptamine release from platelets can hardly be explained by exocytosis if lysophosphatidylcholine is a prerequisite for this mechanism. It cannot, however, be excluded that formation of lysophosphatidylcholine in the membranes of the 5-hydroxytryptamine organelles, e.g. by platelet phospholipase (Silver *et al.*, 1971), might initiate the process of 5-hydroxytryptamine release.

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