Comparison of membrane phospholipid and its fatty acid compositions of the salivary glands from male and female rats

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Introduction

It has been clarified that cyclic nucleotides and calcium ions have a critical role in cellular control mechanisms for the secretory process as second messengers¹⁾. Furthermore, it has become clear that membrane phospholipids participate in the first reaction of secretory mechanisms²⁾. In addition, phospholipids are thought of as a pool of arachidonic acid and that metabolites have many functional action on mammalian cells^{3,4)}. In these respects, salivary glands are useful model system for the studies of receptor mediated secretory mechanism¹⁾. However, there is less information available about the basis analysis of membrane phospholipid composition by considering the difference of each of the three major salivary glands. Therefore, using rat parotid, submandibular and sublingual glands, we analyzed the composition of their phospholipids and fatty acyl chains, and observed that the sublingual gland was slightly different from the other two glands.

Materials and Methods

Ten male and female Sprague-Dawley rats, 9 weeks of age, were used and maintained under similar diet (Oriental MF solid rat chow, Oriental Kobokogyo, Tokyo). After fasting overnight, the rats were sacrificed by bleeding under light ether anesthesia and

then the salivary glands (parotid, submandibular, sublingual) were removed with care to avoid mingling with adipose and connective tissues. Each salivary gland was cut into small pieces and homogenized in 2–3 times volume of ice-cold 0.9% NaCl solution by a Potter-Elvehjem homogenizer.

Total lipids of salivary glands were extracted directly from obtained homogenate by the method of Bligh and Dyer⁵⁾. The first chloroform solution in the procedure of lipid extraction contained 50 µg/ml of BHT (2,6-di-*tert*-butyl-*p*-cresol) to avoid the oxidation of unsaturated fatty acids. The resulting lipids were stored in CHCl₃/CH₃OH (6:1, v/v) under nitrogen at –60°C. Phospholipids were separated from neutral lipids on a silica gel thin-layer chromatography (TLC) plate (Merck). Lipid phosphorus was determined by the procedure of Bartlett⁶⁾, as modified by Marinetti⁷⁾. In order to separate minor phospholipids completely, total phospholipids were applied to the analysis of two dimensional chromatography on Silica Gel H plate containing magnesium acetate (2.5%), using chloroform/methanol/13.5 N ammonia water (65:35:6, v/v) in the 1st dimension, and chloroform/acetone/methanol/acetic acid/water (3:4:1:1:0.5, v/v) in the 2nd dimension⁸⁾. The concentration of each phospholipid on TLC plate was determined by the method of Rouser, *et al.*⁹⁾ The fatty acid methylesters from phospholipids were prepared by the method of Morison and

Smith¹⁰⁾. The areas of phospholipids were scraped off the plates and transmethylated with 10% BF₃/methanol for analysis by gas-liquid chromatography. The methyl-esters were analyzed with a Shimadzu GC-6 gas chromatograph. Each sample was injected onto a glass column (200 cm) packed with 10% diethyleneglycol succinate supported on Chromosorb W (80-100 mesh, Gaschrom Kogyo Co., Tokyo). The column temperature was 185°C and the flow rate of the N₂ carrier gas was 50 ml/min. The unsaturation index is defined as $\Sigma ((\text{number of double bond of each fatty acid}) \times (\% \text{ of each fatty acid}))^{11)}$.

Chemicals were of the highest purity available from commercial sources.

Results and Discussion

As shown in Table 1, the content of membrane phospholipids per tissue wet weight was slightly higher in the sublingual gland than other two glands. This tendency did not change between male and female. In order to analyze phospholipids more in detail, total phospholipids were separated to each one and the composition was compared in the three salivary glands. Phosphatidylcholine and phosphatidylethanolamine were generally major constituents of membrane phospholipids and sphingomyelin, phosphatidylinositol, phosphatidylserine and so on

were present as minor ones. The specific phospholipid for salivary glands was not observed and the phospholipid constituents were similar to the another rat tissues¹²⁾. When the phospholipid compositions were compared between the three salivary glands, they were almost the same in male rat. However, in the female, minor phospholipids, especially phosphatidylinositol and phosphatidic acid, were significantly higher than in the male. This characteristic was observed commonly in the three salivary glands. Phosphatidylinositol has been paid attention because of its rapid turnover, so called "PI effect", in response to various stimuli and this effect is a feature common to not only salivary glands but also to other secreting cells^{13,14)}. Furthermore, phosphatidic acid is one of the metabolite of phosphatidylinositol turnover. Therefore, the obtained results suggest the possibility of different responses to the various stimuli between male and female rat salivary glands.

The biological membrane functions are closely correlated with the physical state of membrane phospholipids¹⁵⁾ and their fatty acyl chains play very important role in it. Therefore, the fatty acid composition of total phospholipids was analyzed in the three major salivary glands. As shown in Table 2, the compositions of fatty acids were very common to the other tissues of rat¹²⁾. Major saturated fatty acids were palmitic

Table 1 Comparison of phospholipid composition in male and female rat salivary glands

	Parotid gland		Submandibular gland		Sublingual gland	
	male (7) ^a	female (3)	male (7)	female (3)	male (7)	female (3)
Total phospholipids ($\mu\text{mol/g}$ of tissue wet weight)	17.0 $\pm$ 1.9	17.5 $\pm$ 0.8	20.2 $\pm$ 2.5	19.8 $\pm$ 0.6	22.1 $\pm$ 1.5	22.7 ^b
Each phospholipid (%)						
phosphatidylcholine	45.9 $\pm$ 4.03	42.2 $\pm$ 2.95	44.4 $\pm$ 1.32	37.9 $\pm$ 1.46	45.3 $\pm$ 1.21	38.6 $\pm$ 1.09
phosphatidylethanolamine	27.3 $\pm$ 1.78	24.4 $\pm$ 1.82	25.9 $\pm$ 1.61	23.7 $\pm$ 0.78	24.7 $\pm$ 0.94	22.4 $\pm$ 1.07
sphingomyelin	7.1 $\pm$ 1.41	7.8 $\pm$ 0.27	7.6 $\pm$ 0.73	7.4 $\pm$ 0.45	7.7 $\pm$ 1.61	6.6 $\pm$ 0.25
phosphatidylinositol	3.4 $\pm$ 0.72	4.9 $\pm$ 1.76	3.4 $\pm$ 0.48	5.0 $\pm$ 0.67	4.9 $\pm$ 1.36	6.4 $\pm$ 0.52
phosphatidylserine	3.6 $\pm$ 0.88	4.3 $\pm$ 1.91	3.9 $\pm$ 1.03	4.3 $\pm$ 0.24	4.5 $\pm$ 1.78	4.7 $\pm$ 0.24
cardiolipin	4.0 $\pm$ 0.86	4.4 $\pm$ 0.23	5.4 $\pm$ 1.31	5.3 $\pm$ 0.41	4.1 $\pm$ 0.47	5.2 $\pm$ 0.06
phosphatidic acid	1.7 $\pm$ 0.27	3.1 $\pm$ 0.06	2.2 $\pm$ 0.85	3.5 $\pm$ 0.38	2.0 $\pm$ 0.83	4.2 $\pm$ 0.30

^a: Values in parentheses represent the number of experiment.

^b: The average of two separate experiments.

Table 2 Comparison of fatty acid composition of total phospholipids in male and female rat salivary glands

Fatty acid (%)	Parotid gland		Submandibular gland		Sublingual gland	
	male (6) ^a	female (2)	male (5)	female (2)	male (4)	female (3)
C14:0	3.9	1.0	2.9	5.3	4.1	7.7
C16:0	27.3	24.0	23.6	24.0	23.0	23.1
C16:1	2.0	1.5	1.9	1.3	2.4	3.0
C18:0	12.6	15.6	13.1	14.9	14.3	14.6
C18:1	11.1	12.6	10.0	10.3	11.0	11.2
C18:2	12.7	16.3	13.8	18.6	10.0	9.7
C20:3	2.5	2.0	4.5	2.6	4.0	2.5
C20:4	18.1	19.5	18.5	15.5	21.1	19.0
C22:6	2.1	1.8	1.3	1.0	1.8	0.9
Others	7.7	5.7	10.4	6.5	8.3	8.3
Unsaturation Index	131.0	141.5	134.8	124.6	140.6	122.5

^a: Values in parentheses represent the number of experiment.

and stearic acids, and arachidonic, oleic and linoleic acids were unsaturated ones. When the compositions were compared between the three glands, the characteristic constitution was observed in the fatty acids of which chain length is eighteen. In parotid and submandibular glands, the compositions of stearic and linoleic acids of total phospholipids were almost same and oleic acid was lower than in the two fatty acids. On the other hand, sublingual gland showed the fatty acid compositions in the order of stearic acid > oleic acid > linoleic acid. These tendencies were also observed regardless of male or female salivary glands. However, the fatty acid composition of total phospholipids is reflected by each phospholipid of which fatty acid shows a very characteristic composition¹²⁾ and the phospholipid composition is also varied depending on the membrane from various organelles^{12,16)}. In order to point out the specific differences in phospholipid and its fatty acid compositions between the three salivary glands, more detailed analysis of phospholipid will be required and is in progress in our laboratory.

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