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**Original Article**

# Enhancement of Acyl-CoA:1-Acyl-*sn*-glycerol-3-phosphocholine Acyltransferase Activity in the Microsomes from Rat Submandibular Gland during the Growth

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*Among the many steps of enzyme reaction for the biosynthesis of phosphatidylcholine, it has been shown that the specific activities of these enzymes are altered during cell growth; therefore, we observed the effects of postnatal growth on a reacylation enzyme, 1-acyl-sn-glycerol-3-phosphocholine acyltransferase, which functions to enrich polyunsaturated fatty acid in phosphatidylcholine in rat salivary glands. The wet weights were three- and two-times increased during growth from 3 to 9 weeks old in rat submandibular and parotid glands, respectively. In microsomes from 3-week-old rat submandibular and parotid glands, and liver, 1-acyl-sn-glycerol-3-phosphocholine acyltransferase activities were higher in the order, liver＞submandibular≧parotid glands. The specificities for acyl-CoAs were very similar; however, in 10-week-old rats, the activity from submandibular microsomes was 4- to 7-fold higher than in 3-week-old rats. In this enhancement of specific activity, $K_m$ values for arachidonoyl-CoA (20：4-CoA) did not change; on the contrary, specific activities of this enzyme in the parotid gland and liver were hardly changed during growth. These results suggest that the tissue-specific enhancement of 1-acyl-sn-glycerol-3-phosphocholine acyltransferase activity may occur due to the induction of its protein, which may play a specific role in the growth of the submandibular gland.*

Key words: 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase activity, acyl-CoA specificity, phosphatidylcholine, salivary gland

## INTRODUCTION

It is not only phospholipids, which have long been recognized as important structural components of cells, but also specific biologies associated with specific lipids and their metabolites have been gathering much attention over the past decade[1,2]. Phosphatidylcholine is a major phospholipid presents in mammalian cell membranes as a class of glycerophospholipids and an important constituent of serum lipoproteins in mammalian tissues[3]. Recently, phosphatidylcholine has been gathering much attention due to it not only being a major structural component of membrane bilayers but also its functional roles such as in apoptosis[4], and involvement with eicosanoids[5] and lysophospholipids[6,7]. Among the many steps in the enzyme reaction leading to the biosynthesis of phosphatidylcholine, research into the regulatory mechanisms of activities in physiological functions has made considerable progress, and several reports have shown that the specific activities of the involved enzymes are altered during the period of development[3,8]. In rat salivary glands,(parotid and submandibular), proliferated by chronic administration of β-adrenergic agonist, the specific activity and substrate specificity of acyl-CoA: 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase, which contributes to maintaining the asymmetric distribution of fatty acyl groups in phosphatidylcholine, were markedly changed[9,10]. In this paper, therefore, we observed the effects of postnatal development on 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase activity in rat salivary glands.

## MATERIALS AND METHODS

### 1. Animals

Male Sprague-Dawley rats (3-10 weeks old) were maintained ad libitum on Oriental MF solid chow (Oriental Yeast Co., Tokyo) and water. The present experimental protocol was approved by the Animal Ethics Committee of Asahi University (No.05-003)[11].

After fasting overnight, all rats were killed by bleeding under light diethyl ether anesthesia. Immediately following this, the parotid and submandibular glands and liver were removed and carefully trimmed of connective tissue, blood vessels, and the capsule. Liver was perfused by saline solution. The obtained gland and liver tissues were stored at −60℃ until use.

### 2. Preparation of microsomes

All procedures were carried out at 0-4℃. Frozen tissue was thawed, cut into small pieces with a McIlwain Tissue Chopper(Mickel Laboratory Engineering Co., Gomshall, Surrey, UK), and then homogenized with a Potter-Elvehjem Teflon pestle homogenizer in 0.1 M Tris-HCl buffer (pH 7.2) containing 0.25 M sucrose. The microsomes were obtained as a precipitate of centrifugation (105,000×*g*, 60 min), as described previously[12]. The resulting pellet was suspended at approximately 10 mg microsomal protein per ml in 0.1 M Tris-HCl buffer (pH 7.2) without sucrose, using a glass pestle homogenizer, and was stored at −85℃ until use.

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3. Assay of acyltransferase activity

1-Acyl-*sn*-glycerol-3-phosphocholine was prepared from egg yolk phosphatidylcholine by hydrolysis with snake venom phospholipase $A_2$[13]. Linoleoyl and arachidonoyl-CoAs were synthesized by means of the coupling reaction of acylchloride derived from fatty acids and coenzyme A[14,15]. Other acyl-CoAs used were of the highest grade available from commercial sources, as described[12].

Acyl-CoA : 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase activities were measured spectrophotometrically using 5, 5'-dithiobis (2-nitrobenzoic acid)[13,16]. The reaction mixture contained 20 μM acyl-CoA, 150 μM 1-acyl-glycerophosphocholine, 1 mM DTNB, and 100 μg/m*l* of microsomal protein in 90 mM Tris-HCl buffer (pH 7.2), and incubation was carried out at 25℃ in duplicate.

4. Other methods

The protein concentration was determined according to the method of Lowry *et al*.[17] or with the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) according to Bradford[18], using bovine serum albumin as the standard.

## RESULTS AND DISCUSSION

According to the growth of rats, the wet weights of their salivary glands were increased (Fig.1), and the increasing rates in parotid and submandibular glands between 3- and 9-week-old rats were 1.8- and 3.1-fold, respectively. It is generally thought that membrane phospholipid biogenesis is enhanced in the growth stage. Indeed, lysophospholipid acyltransferase activity was increased 2-fold in the microsomes of parotid glands proliferated by isoproterenol treatment[9]. Conversely, the same enzyme activity in the microsomes from the proliferated submandibular gland was decreased[10,16]. In mammalian tissues, the reacylation of lysophospholipids plays an important role in establishing molecular species of membrane phospholpids and regulating the free polyunsaturated fatty acids level[13,19]. During the growth of rat salivary glands following birth, on the other hand, the maturation of receptor-linked secretory function develops extensively in 3 weeks[20,21]. Since acyl-CoA:1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase is thought to be one of the key enzymes involved in the regulation of membrane phospholipid constitutions, the activity was compared in growing rat salivary glands postnataly. As shown in Table 1, the specific activities in the microsomes from the parotid gland were almost the same between 3- and 9-week-old rats. Under optimal conditions, acyl-CoA specificities were also compared, but they did not show any change. These tendencies in the specific activity and acyl-CoA specificity were observed in the liver microsomes (Table 2). On the contrary, as shown in Table 3, 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase activities were about 4- to 7-fold higher in 9-week-old rats than in 3-week-old rats in the subman-

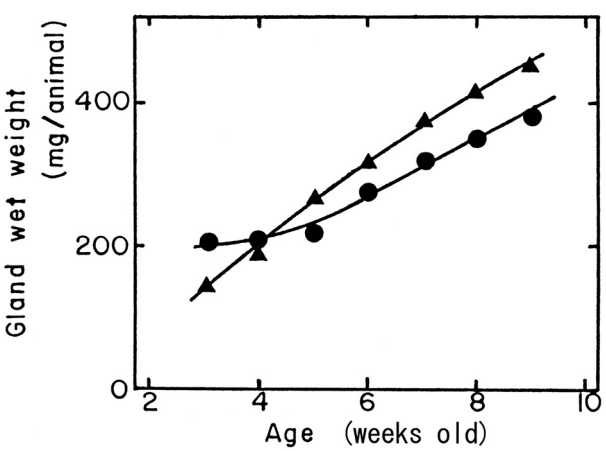


Fig. 1 Changes in tissue wet weights of rat salivary glands
●, parotid; ▲, submandibular

Table 1 Acyl-CoA specificities of 1-acyl-sn-glycerol-3-phosphocholine acyltransferase in microsomes from 3- and 9-week-old rat parotid glands

| | 3 weeks old | | 9 weeks old | | |
|---|---|---|---|---|---|
| Acyl-CoA | Specific activity (A) | Relative activity | Specific activity (B) | Relative activity | B/A |
| 16:0*-CoA | 3.0 | 1.0 | 3.5 | 1.0 | 1.2 |
| 18:0-CoA | 0.7 | 0.2 | — | — | — |
| 18:1-CoA | 17.7 | 5.9 | 14.6 | 4.2 | 0.8 |
| 18:2-CoA | 6.3 | 2.1 | 9.2 | 2.6 | 1.5 |
| 20:4-CoA | 9.9 | 3.3 | 10.3 | 2.9 | 1.0 |

The specific activities (A and B) are represented as nmol/min per mg of microsomal protein.
*Acyl chain; chain length:number of double bonds.

Table 2 Acyl-CoA specificities of 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase in microsomes from 3- and 9-week-old rat liver

| | 3 weeks old | | 9 weeks old | | |
|---|---|---|---|---|---|
| Acyl-CoA | Specific activity (A) | Relative activity | Specific activity (B) | Relative activity | B/A |
| 16:0*-CoA | 8.5 | 1.0 | 6.5 | 1.0 | 0.8 |
| 18:0-CoA | 6.8 | 0.8 | — | — | — |
| 18:1-CoA | 36.1 | 4.3 | 37.1 | 5.7 | 1.0 |
| 18:2-CoA | 23.0 | 2.7 | 25.3 | 3.9 | 1.1 |
| 20:4-CoA | 41.6 | 4.9 | 33.1 | 5.1 | 0.8 |

The specific activities (A and B) are represented as nmol/min per mg of microsomal protein.
*Acyl chain; chain length:number of double bonds.

Table 3 Acyl-CoA specificities of 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase in microsomes from 3- and 9-week-old rat submandibular glands

| | 3 weeks old | | 9 weeks old | | |
|---|---|---|---|---|---|
| Acyl-CoA | Specific activity (A) | Relative activity | Specific activity (B) | Relative activity | B/A |
| 16:0*-CoA | 2.8 | 1.0 | 13.9 | 1.0 | 5.0 |
| 18:0-CoA | 2.1 | 0.8 | 9.1 | 0.7 | 4.3 |
| 18:1-CoA | 10.4 | 3.7 | 51.5 | 3.7 | 5.0 |
| 18:2-CoA | 3.8 | 1.4 | 28.2 | 2.0 | 7.4 |
| 20:4-CoA | 6.2 | 2.2 | 39.4 | 2.8 | 6.4 |

The specific activities (A and B) are represented as nmol/min per mg of microsomal protein.
*Acyl chain; chain length:number of double bonds.

dibular glands. However, acyl-CoA specificities did not change during growth. The phosphatidylcholine composition in the submandibular gland increases, but the fatty acid composition of phosphatidylcholine shows little change along with growth[22]. The characteristics of acyltransferase might explain the part of the results obtained by the analyses of phosphatidylcholine from the submandibular gland. In liver microsomes, the highest activity between the various acyl-CoAs used in this paper was observed in arachidonoyl-CoA. On the contrary, in the salivary glands, the activity for oleoyl-CoA was the highest and followed in the order of oleoyl-CoA＞linoleoyl-CoA＝arachidonoyl-CoA. These specificities were similar between submandibular and parotid gland microsomes. The reacylation enzyme reported in this paper functions to incorporate polyunsaturated fatty acids into the C-2 position of phosphatidylcholine, the largest constituent of membrane phospholipids[1,3]. Indeed, 62% of the C-2 position of phosphatidylcholine are oleic, linoleic, and arachidonic acids[22]. The acyl-CoA specificities of acyltransferase that showed the higher activities in oleoyl-CoA, linoleoyl-CoA, and arachidonoyl-CoA may play an important role in determining the distribution of the fatty acid pattern in phosphatidylcholine.

In order to clarify whether the enhancement of acyltransferase activity observed in submandibular gland microsomes is accompanied with changes of enzyme characteristics, the effect of the concentration of arachidonoyl-CoA on acyltransferase activity in the submandibular glands from 3 and 9 weeks old was investigated. According to the analysis of Lineweaver-Burk plots[23], $V_{max}$ using arachidonoyl-CoA was 66.7 nmol/min/mg of protein in 10-week-old rat submandibular microsomes. On the other hand, in 3-week-old rats, $V_{max}$ was 20.4 nmol/min/mg of protein. When $V_{max}$ values are compared according to growth, the enzyme activity in 9-week-old rat submandibular gland microsomes was about 3.3 fold higher than in 3-week-old rats. However, the apparent $K_m$ values for arachidonoyl-CoA were very similar between the two different aged gland microsomes, at 3.7μM. These observations suggest that the enhancement of the acyltransferase activity may be due to the increase of enzyme protein, not to the change of enzyme properties, such as activation.

From the results obtained here, it is suggested that the changes of 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase activity are not associated directly with cell growth in rat salivary glands, but specific development in submandibular growth. By the chronic administration of β-adrenergic agonist, parotid and submandibular cell growth proliferated markedly, and the specific activity and substrate specificity of acyl-CoA:1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase were changed[9,10]. However, the tendency in the alteration of enzyme activity was different between parotid and submandibular glands, in spite of the apparent growth. This tissue-specific enhancement of acyltransferase activity may occur in the period of receptor maturation and be due to the induction of enzyme protein, which may play a specific role in the submandibular gland.

Lysophospholipids, the substrate for reacylation enzymes observed in this report, regulate membrane fusion by protein-lipid interplay[24], and partially participate in saliva secretion accompanied by exocytosis[25]. Therefore, the reacylation enzymes might also regulate the function of salivary glands.

On the other hand, there are also many other important reacylation enzymes, such as 1-acyl-*sn*-glycerol-3-phosphoinositol acyltransferase, 1-acyl-*sn*-glycerol-3-phosphoethanolamine acyltransferase, 1-acyl-*sn*-glycerol-3-phosphoserine acyltransferase, and 1-acyl-*sn*-glycerol-3-phosphate acyltransferase, etc. The effects of these enzyme activities on the growth of salivary glands remain to be clarified. Further studies will be necessary to clarify the reason for changes in acyltransferase activity in proliferated salivary glands.

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# ラット顎下腺ミクロソームのアシル-CoA：1-アシル-*sn*-グリセロール-3-ホスホコリンアシルトランスフェラーゼの成長に伴う活性上昇

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ホスファチジルコリンは，生体膜リン脂質の主要構成成分であり，エイコサノイド前駆体の多価不飽和脂肪酸の貯蔵部位である．その脂肪酸導入にはリン脂質再アシル化系酵素の一つである1-アシル-*sn*-グリセロール-3-ホスホコリンアシルトランスフェラーゼが関与する．そこで，ラット唾液腺の成長に伴う本酵素の活性変化について検討した．ラット顎下腺，耳下腺の湿重量は，3から9週齢に成長するにしたがってそれぞれ3および2倍に増加した．3週齢の酵素の比活性は，肝＞顎下腺≧耳下腺の順であり，アシル-CoAに対する基質特異性は3組織間でほとんど変わらなかった．しかし，3週齢から10週齢に成長した顎下腺では，アシル-CoAに対する基質特異性，$K_m$値は変化することなく，酵素活性が4～7倍に上昇した．この変化は耳下腺，肝では観察されなかった．これらの結果は，顎下腺の生後成熟過程に多価不飽和脂肪酸導入酵素が特異的に寄与している可能性が示唆された．

キーワード：1-アシル-*sn*-グリセロール-3-ホスホコリンアシルトランスフェラーゼ，アシル-CoA基質特異性，ホスファチジルコリン，唾液腺

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