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PHOSPHOLIPASE C ACTIVITY OF RAT TISSUES[+]

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SUMMARY

Phospholipase C activity was detected in rat tissues in vitro by measuring the conversion of [1-^{14}C]dioleoylphosphatidylcholine to [^{14}C]diglyceride and [^{14}C]monoglyceride. Adipose tissue, brain, diaphragm, duodenum, heart, ileum, jejunum, kidney, liver, lung, skeletal muscle and spleen were homogenized and incubated at pH 4.8, in vitro. All of the tissue homogenates converted aqueous dispersions of [^{14}C]phosphatidylcholine to ^{14}C-labeled diglyceride and monoglyceride, demonstrating the presence of phospholipase C. This type of phospholipase C has been previously described only in bacteria. These studies demonstrate for the first time the presence of Phospholipase C in a wide spectrum of rat tissues.

INTRODUCTION

Phospholipase C (EC 3.1.4.3), which hydrolyzes the ester bond of phosphoglycerides between diacylglycerol and the substituted phosphoric acid group, has previously been described only in bacteria (1-11) although a phosphatidylinositol-specific phospholipase C has been demonstrated in several mammalian tissues including brain, kidney, liver, intestinal mucosa and platelets (12-23). Recently, we demonstrated phospholipase C activity in a soluble protein fraction prepared from rat liver lysosomes (24). In contrast to bacterial phospholipases C, liver lysosomal phospholipase C does not require divalent cations and is not inhibited by EDTA; it has an acid pH optimum while the pH optimum of bacterial phospholipases C is either neutral or slightly alkaline (1-11). Our findings suggested the possibility that phospholipase C might also be present in other tissues. If so, phosphatidylcholine could be degraded by either

[+]Abbreviations used: PC, phosphatidylcholine; LPC, lysophosphatidylcholine; P'Lipase A, phospholipase A; P'Lipase C, phospholipase C; DG, diglyceride; DL, diglyceride lipase; LPL, lysophospholipase; GPC, glycerophosphocholine; MG, monoglyceride; ML, monoglyceride lipase; G, glycerol: FA, fatty acid.

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the phospholipase A pathway or by the phospholipase C pathway as shown below:

$$PC \xrightarrow{P'Lipase\ A} LPC \xrightarrow{LPL} GPC + 2\ FA \qquad (1)$$

$$PC \xrightarrow{P'Lipase\ C} phosphocholine + DG \xrightarrow{DL} MG \xrightarrow{ML} G + 2\ FA \qquad (2)$$

In this publication, the activity of phospholipase A and C has been examined in rat tissues in vitro using [1-^{14}C]dioleoylphosphatidylcholine. Our results present qualitative evidence for the presence of phospholipase C in all of the rat tissues examined.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain were fasted overnight, killed by cervical dislocation and the following tissues were removed at once, rinsed and chilled in cold buffer: adipose, brain, diaphragm, heart, kidney, liver, lung, small intestine, skeletal muscle and spleen. The tissues were homogenized for 10-20 sec in cold 0.25M sucrose containing 5 mM Tris-HCl (pH 7.4) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). In the case of adipose tissue, the floating fat layer in the homogenate was removed and discarded. Protein was determined by the method of Lowry et al (26), and the homogenates were stored frozen at -60° until use.

Phospholipase C activity was determined by assaying the release of [^{14}C]-diglyceride and [^{14}C]monoglyceride from sonicated dispersions of [1-^{14}C]dioleoyl-phosphatidylcholine (Applied Science Laboratories, College Park, PA) as previously described (24,25). The incubation conditions are given in the respective legends. Diglyceride, monoglyceride, fatty acid, phosphatidylcholine and lysophosphatidyl-choline were measured as described previously (25). The lipids were located by radioscanning with a Panax scanner (Panax Instruments, Redhill, Surrey, England) scraped into vials and counted by liquid scintillation as previously described (24,25).

RESULTS AND DISCUSSION

As shown in Table 1, all tissues were active in the formation of ^{14}C-labeled diglyceride and monoglyceride at pH 4.8. In most tissues, with the exception of small intestine, monoglyceride exceeded diglyceride, accounting for more than 50 percent of the total. This is apparently due to the presence in these tissues of an acid diglyceride lipase; we have previously presented evidence supporting this interpretation in rat liver lysosomes (24). In the small intestine, diglyceride is present in larger amounts than monoglyceride in duodenum and jejunum, while diglyceride and monoglyceride are equal in ileum.

Table 1 Hydrolysis of [1-^{14}C]dioleoylphosphatidylcholine by Rat Tissue Homogenates

Tissue	Products Formed			
	Lysophosphatidylcholine	Monoglyceride	Diglyceride	Fatty Acid
Adipose	2.75 ± 0.49	0.67 ± 0.10	0.30 ± 0.04	2.14 ± 0.55
Brain	0.70 ± 0.34	0.41 ± 0.05	0.10 ± 0.02	0.87 ± 0.02
Diaphragm	0.18 ± 0.32	0.21 ± 0.13	0.06 ± 0.01	0.41 ± 0.01
Heart	0.27 ± 0.24	0.23 ± 0.13	0.07 ± 0.05	0.56 ± 0.08
Kidney	1.40 ± 0.35	0.44 ± 0.03	0.13 ± 0.08	1.47 ± 0.42
Liver	1.48 ± 0.15	0.53 ± 0.15	0.18 ± 0.03	1.54 ± 0.38
Lung	2.16 ± 0.90	0.84 ± 0.15	0.36 ± 0.12	3.06 ± 0.25
Skeletal muscle	0.08 ± 0.08	0.04 ± 0.04	0.01 ± 0.01	0.13 ± 0.02
Small intestine:				
Duodenum	1.24 ± 0.73	1.68 ± 0.53	2.25 ± 0.75	19.6 ± 6.24
Jejunum	1.19 ± 0.81	1.60 ± 0.24	1.97 ± 0.56	16.8 ± 1.82
Ileum	1.24 ± 0.61	1.90 ± 0.25	1.89 ± 0.47	17.2 ± 2.22
Spleen	3.17 ± 0.26	0.73 ± 0.19	0.49 ± 0.16	4.34 ± 0.77

The incubation mixtures contained 50 mM sodium acetate (pH 4.8), 4.5 x 10^{-5}M [1-^{14}C]dioleoylphosphatidylcholine, specific activity 30 mCi/mmol, and 200 µg of homogenate protein in a total volume of 0.200 ml. The mixture was incubated for 60 min at 37°; the reaction was stopped with 20 volumes of chloroform/methanol, 2/1 by volume and total lipid extracts prepared as described previously (24). The results are the mean ± 1 std. deviation of 3 separate experiments and represent the nmoles of product produced per mg protein per hr above that found in a control incubated without protein.

Phospholipase C activity of rat tissues may be estimated from the three experiments as monoglyceride plus diglyceride. Small intestine was most active of rat tissues; formation of monoglyceride and diglyceride ranged from 3.50 to 3.95 nmoles mg^{-1} hr^{-1} in duodenum, jejunum and ileum. The small differences in phospholipase C activity between these portions of the small intestine were not statistically significant. Phospholipase C activity in spleen, lung and adipose tissue was 1.19, 1.19 and 0.96 nmol mg^{-1} hr^{-1}, respectively, while the activity in liver, kidney and brain was 0.71, 0.57 and 0.51 nmol mg^{-1} hr^{-1}. Muscle tissues had the lowest levels of phospholipase C activity ranging from 0.04 to 0.30 nmol mg^{-1} hr^{-1}.

Phospholipase A activity of rat tissues has been estimated by the formation of [^{14}C]lysophosphatidylcholine. As shown in Table 1, the highest levels of phospholipase A activity were found in adipose tissue, 2.75 nmol mg^{-1} hr^{-1}, and spleen, 3.17 nmol mg^{-1} hr^{-1}. Phospholipase A activity was intermediate in lung, liver, kidney and brain; 2.16, 1.48, 1.40 and 0.70 nmol mg^{-1} hr^{-1}, respectively. The formation of [^{14}C]lysophosphatidylcholine was much lower in the homogenates of heart, diaphragm and skeletal muscle; 0.27, 0.18 and 0.08 nmol mg^{-1} hr^{-1}, respectively.

It should be noted that estimates of phospholipase C and phospholipase A activity based on the rate of generation of ^{14}C-labeled partial glycerides and lysophosphatidylcholine cannot be taken as accurate quantitative measures of these pathways since the products are subject to removal by monoglyceride lipase and lysophospholipase as shown above (reactions 1 and 2). If the activity of lysophospholipase and monoglyceride lipase is not rate-limiting in the respective pathways, assessment of their contribution to the overall process cannot readily be estimated. Furthermore, although the production of diglyceride and fatty acid was progressive with incubation times of 20, 40 and 60 minutes, monoglyceride often did not increase progressively during the incubation (data not shown). Thus, these results should be regarded as minimum estimates of the activity of phospholipases A and C.

These studies are the first to show that phospholipase C is not limited to bacteria but is present in all of the rat tissues examined. The phospholipase C activities measured in these experiments must be regarded as minimum values since monoglyceride can be removed by the further action of monoglyceride lipase as noted above. Nevertheless, the results indicate in a qualitative way that phospholipase C activity is present in adipose tissue, brain, diaphragm, duodenum, heart, ileum, jejunum, kidney, liver, lung, skeletal muscle and spleen.

The physiological significance and role of phospholipase C in these rat tissues is not presently clear. We have previously shown that acid phospholipase C is lysosomal in rat liver (24). Lysosomal phospholipase C may be of importance

in the degradation of lipoprotein-derived phospholipids which enter cells by adsorptive endocytosis (27). Phospholipase C represents an alternative pathway for phospholipid degradation which does not result in the generation of lysophosphoglycerides, compounds with well-known detergent properties which can cause lysis of lipid bilayer membranes. The presence of this enzyme might serve to preserve the integrity of the lysosomal membrane during lysosomal degradation of phosphoglycerides since the products of phospholipase C action would be expected to be less damaging to membranes than the lysophosphoglycerides produced by phospholipase A (28-31). Finally, the substantial activity of acid phospholipases in the small intestinal tissues (duodenum, jejunum and ileum) is unexpected and raises a question as to the possible role of these enzymes in the intestinal metabolism of phosphoglycerides. Further studies will be necessary to define the quantitative importance and the physiological role of phospholipase C in mammalian tissues.

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