EFFECT OF PHOSPHOLIPASE A, C, AND D ON SUGAR AND AMINO ACID TRANSPORT IN RAT UTERUS

Hiroshi KOGO and Yoshio AIZAWA
Department of Pharmacology, Tokyo College of Pharmacy, Shinjuku-ku, Tokyo, Japan

Received for publication November 19, 1971

It is widely known that estrogen can stimulate transport of both sugars and amino acids into rat uterus (1-6). This estrogen effect on transport may reflect the anabolic activity of the hormone in uterus but its mechanism is little known. Phospholipid in tissues is an essential component of cell membrane which is composed mainly of lipid and protein, though the basic arrangements of these two components are not clear. On the other hand, phospholipid in rat uterus is the major organic component that undergoes rapid acceleration of metabolism and increases at an early phase after estrogen administration (7). It can be presumed that cell membrane in an organ plays an important role in the transport of substrates. Recently, the role of phospholipid on sugar transport was reported and it was observed that the rate of intracellular 3-0-methylglucose space in the castrated rat uterus increased by the addition of phospholipid (8).

On the basis of these results, it seemed of interest to study the role of phospholipid on sugar and amino acid transport in rat uterine cell membrane, and such an examination was carried out by using phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4), phospholipase C (phosphatidylcholine-phosphohydrolase, EC 3.1.4.3), and phospholipase D (phosphatidylcholine phosphatidohydrolase, EC 3.1.4.4).

This report shows that the phosphate position and/or part of basic groups of phospholipid in a membrane tissue may be playing a significant role on sugar and amino acid transport in rat uterus that was increased by estrogen.

METHODS

Wistar strain female rats, weighing approx. 100 g, were used 3 weeks after ovariectomy. Ten μg of 17β-estradiol in 0.1 ml of sesame oil was injected subcutaneously to castrated rats while controls received the vehicle alone. The animals were sacrificed by decapitation after 4 hr. 3-0-Methyl (14C)-D-glucose and α-aminoisobutyric-(1-14C) acid were obtained from the New England Nuclear Corporation. Phospholipase C (from Clostridium welchii) was obtained from Sigma Chemical Co., phospholipase A (from Crotalus terr. terr.) from Boehringer-Mannheim, phospholipase D (from cabbage) also from Boehringer-Mannheim. Treatment was carried out by preincubation for 30 min at 25°C with the enzyme (4 units per ml of preincubation medium) in one flask which contained uteri from each of 5 animals of estrogen-treated or untreated rats, and phospholipase-untreated controls with a medium...
alone without the enzyme. The preincubation medium was as follows: For phospholipase A and C; 0.9 % NaCl (containing 0.01 M CaCl₂) buffered to pH 7.5 with 0.025 M Tris-HCl and for phospholipase D, 0.9 % NaCl (containing 0.01 M CaCl₂) buffered to pH 5.9 with 0.1 M acetate. Incubation with Krebs-Ringer bicarbonate solution for sugar and amino acid transport was carried out by the same method as the previous work (8). Protein was determined by the method of Lowry and Rosebrough (9) and phosphorus content of phospholipid by the method of Chen et al. (10) after digestion with perchloric acid.

RESULTS

Fig. 1 shows the effect of phospholipase A, C, and D on the uptake of 3-0-methyl(¹⁴C)-D-glucose (3-0-MG) into rat uterus, which increased by approx. 40-50 % after estrogen injection. Phospholipase A treatment did not influence the uptake of 3-0-MG in both estrogen-treated and -untreated control. While it was recognized that phospholipase C and D treatment decreased the 3-0-MG uptake in the estrogen-treated group to nearly that of the untreated control, that in the castrated control group was not greatly changed by the enzyme treatment.

Similar to 3-0-MG, effect of phospholipase A, C, and D treatment on the uptake of α-aminobutyric-(¹⁴C) acid (AIB) was studied on rat uterus (Fig. 2). Effect of estrogen on the uptake of AIB into rat uterus was stronger than that on 3-0-MG. Effect of phos-

![Fig. 1](image-url)  
**Fig. 1.** Effect of phospholipase A, C, and D on uptake of 3-0-methyl (¹⁴C)-D-glucose in rat uterus.  
Histogram shows the of uptake of 3-0-methyl-D-glucose in control and phospholipase (PLase) treated sample. Height of the bar represents an average of 5 animals and the vertical line through the bar represents standard error of the mean.  
Estrogen (E₂) treated group was given 10 μg of 17β-estradiol in sesame oil for castrated (Cast.) rat and the rats sacrificed at 4 hr.  
Uteri prepared from 5 animals each of estrogen-treated and non-treated rats were treated for 30 min at 25 °C with respective phospholipase of 4 units/ml of incubation medium in one flask.  
* P<0.05  
** P<0.1
Fig. 2. Effect of phospholipase A, C, and D on uptake of α-Aminoisobutyric-(1-^{15}C) acid in rat uterus.

Height of the bar shows the rate of uptake of α-aminoisobutyric acid in control and phospholipase (PLase) treated sample and represents average of 5 animals and the vertical line through the bar represents standard error of the mean.

Experimental condition was the same as given in Fig. 1.

* P<0.001

<table>
<thead>
<tr>
<th>Table 1. Effect of phospholipase C on rat uterus.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid content in uterus (μ mole P/mg protein)</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Untreated rat uterus</td>
</tr>
<tr>
<td>Phospholipase C treated uterus</td>
</tr>
</tbody>
</table>

Two rats were used in this experiment, and excised uterus was divided into equal parts from the horn. In each of 2 flasks, 2 segments each from two different animals, making 4 segments from two rats, were incubated with or without phospholipase C for 30 min at 25°C in the medium of phospholipase treatment. At the end of incubation, phospholipid in the uterus was extracted with 1 ml each of EtOH, CHCl₃, and ether.

Phospholipid content in the uterus and phosphorus liberated into the medium were determined by the method of Chen et al. (10) after digestion with perchloric acid.

Phospholipase A treatment on the uptake of AIB was hardly influenced in either the estrogen-treated or the -untreated control but AIB uptake of the enzyme-treated group showed a tendency to increase slightly compared to the enzyme-untreated group. Treatment with phospholipase C and D inhibited the uptake of AIB into uterus, likewise that of 3-0-MG. In particular, it was recognized that phospholipase C treatment markedly influenced on the uptake of 3-0-MG and AIB into rat uterus. Tabulated values in Table I show the amount of phospholipid in uterine tissue and of phosphorus liberated into the medium from the uterus of enzyme-treated and untreated rats. Phospholipid content in rat uterus decreased to 55% of the control uterus under phospholipase treatment. In addition, a larger amount of phosphorus was detected in phospholipase C-treated medium than in enzyme-untreated medium.
DISCUSSION

It is reported that phospholipase of hydrolytic enzymes can specifically hydrolyze phospholipid which is a component of the membrane (11–13). Phospholipases provide an obvious tool for producing selective destructive changes in the cell membrane under generally mild conditions. They have been known to alter the membrane properties of tissues, cells, and their subcellular fractions, such as thyroid slices (14), intestinal mucosa (15), erythrocytes (16), adipose cells (17), mitochondrial fraction (18, 19), and microsomal fraction (20).

Results reported in this paper shows that sugar and amino acid uptake in the uterus from estrogen-injected rats decreased by pretreatment with phospholipase C and D but the uptake in hormone-untreated castrated uterus was hardly affected. Phospholipase A treatment did not inhibit the uptake of sugar and amino acid in both estrogen-treated and untreated ovariectomized rat uterus. Phospholipase C is the enzyme which specifically catalyzes hydrolytic cleavage of phospholipids, mainly phosphatidylcholine and phosphatidylethanolamine, to diglyceride and corresponding phosphoryl products. On the other hand, it is known that phospholipase D catalyzes hydrolytic cleavage of the terminal phosphate diester bond in glycerophosphatides containing choline, ethanolamine, serine, or glycerol, with the formation of phosphatidic acid. Both phospholipases C and D catalyze hydrolytic cleavage of phosphate diester bond in phospholipid. From these facts and the present experimental results, it can be assumed that the phosphate diester bond of phospholipid in a cell membrane is an essential part for sugar and amino acid transport increased by estrogen. Chapman et al. (21) showed in a study on molecular interactions in erythrocyte membranes using NMR that N+CH3 protons of phosphatidylcholine in biological membrane have freedom of molecular motion. It is thought that the effect of phospholipase C and D on transport is the induction of conformational changes on the surface of cell membranes in uterine tissues or that they provide changes in the environment of the enzyme system involved in the transport. Effect of phospholipase C and D on sugar and amino acid was notably observed in estrogen-treated groups. This result may be related to the stimulation of phospholipid metabolism by estrogen administration (7, 8, 19, 22). It is probable that phospholipid plays an important role in the mechanism of estrogen action on sugar and amino acid transport in rat uterus.

The effects of estrogen on the metabolism of phospholipids in rat myometrial membrane and influence of phospholipase C on phospholipid metabolism in rat uterus are now under research in this laboratory.

SUMMARY

The role of phospholipid in uterine cell membrane on sugar and amino acid transport into rat uterus was studied by the use of phospholipase A (EC 3.1.1.4), phospholipase C (EC 3.1.4.3), and phospholipase D (EC 3.1.4.4). Non-metabolizable 3-O-methyl(14C)-D-glucose (3-O-MG) and L-aspartic acid(14C) acid (AIB) were used for this examination. Results are as follows:
1. Treatment of rat uterus with phospholipase A did not influence the uptake of 3-0-MG into the uterus.

2. Treatment of rat uterus with phospholipase C or D decreased the 3-0-MG uptake in estrogen-treated group nearly to the untreated control level but that in the castrated control group was not greatly changed.

3. Treatment of rat uterus with phospholipase A hardly affected the uptake of AIB into the uterus.

4. Treatment of rat uterus with phospholipase C or D inhibited the uptake of AIB into the uterus in estrogen-treated group more than that in untreated group.

These results show that the phosphate position and/or part of basic group of phospholipid in the membrane tissue may be playing a significant role on sugar and amino acid transport in rat uterus increased by estrogen.

REFERENCES
2) SPAZIANI, E. AND GUTMAN, A.: Endocrinol. 76, 470 (1965)
3) SPAZIANI, E. AND SUDDICK, R.P.: Endocrinol. 81, 205 (1967)