ROLE OF PHOSPHOLIPID IN THE EFFECT OF ESTRADIOL ON SUGAR TRANSPORT IN RAT UTERUS

Hiroshi KOGO and Yoshio AIZAWA

Department of Pharmacology, Tokyo College of Pharmacy, Shinjuku-ku, Tokyo, Japan

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In 1961, one of the authors (1) demonstrated that estrogen stimulated the biosynthesis of phospholipids in the ovariectomized rat uterus and phospholipid metabolism constitutes an unusually sensitive indicator of early estrogen action. Recently, Roskoski and Steiner reported that estrogen injection increases the initial rate of 3-O-methylglucose transport into uterine cells and this transport takes place by means of a specific mobile carrier transport system (2).

Phospholipid is an essential component of biological membrane (3, 4) and it has been clarified that phospholipid affects various enzymic reactions (5–10). Membrane lipid is also of interest in this regard as it may play an important role in transport of a substance through cell membrane.

This present report is an investigation as to whether or not sugar transport into uterine cells, which is accelerated by estrogen administration, is affected by phospholipids in vitro. It was observed that sugar transport in the spayed rat uterus increased with the addition of phospholipid.

METHODS

Unless otherwise specified, Wistar strain female rats, weighing approx. 100 g, were spayed the same day under ether anesthesia and maintained for 3 weeks, then subcutaneous injections of 10 μg 17β-estradiol in 0.1 ml of sesame oil or the vehicle alone. They were then sacrificed by decapitation at an indicated time. The 3-O-[14C]methyl-D-glucose (16.5 mCi/m mole) and D-(1-3H)-sorbitol (6.7 Ci/m mole) were obtained from New England Nuclear Corporation. Incubation procedures carried out were similar to the method of Roskoski and Steiner (2), at the end of which time, the uterus horns were washed twice with 5 ml of Krebs-Ringer bicarbonate solution (pH 7.4). Each horn was incised longitudinally, blotted on dry filter paper, weighed on torsion balance, and transferred to microtest tube. After tubes had been boiled for 15 min, uterine tissues were homogenized with 1 ml of 5%, trichloroacetic acid in an all glass homogenizer and the uterine homogenates were centrifuged at 800 g for 15 min. From one-tenth to three-tenths ml aliquot of the clear supernatant was completely evaporated in a counting vial. The residue was dissolved in 0.1 ml of distilled water and five ml of scintillation solution (4 g of 2,5-diphenyloxazole plus 0.1 g of 1,4-bis-2-(methyl-5-phenyl-oxazolyl) benzene per liter of toluene plus 300 ml...
of ethanol. The radioactivity was counted by a liquid scintillation spectrometer. Results are expressed as a space by the definitions of Roskoski and Steiner. Phospholipids were prepared from female rats liver by the method of Hanahan et al. (11). Lipid was suspended in the incubation medium by teflon homogenizer and used for experiment.

RESULTS

Fig. 1 shows sugar transport after estrogen treatment. The upper line shows the rate of 3-O-methyl-D-glucose space, the middle line the rate of D-sorbitol space, and the lower line the rate of intracellular 3-O-methyl-D-glucose space. It was observed that the rate of 3-O-methyl-D-glucose uptake increased by estrogen administration but the rate of D-sorbitol uptake into rat uterus was little changed by estrogen injection. The rate of intracellular 3-O-methyl-D-glucose uptake was recognized to be an obvious increase after 4 hr of hormone treatment. The relation between incubation time and the rate of intracellular 3-O-methyl-D-glucose uptake on uterus from estrogen-treated (4 hr) and untreated ovariectomized rat are shown in Fig. 2. Maximal response of estrogen effect was observed by incubation time to be between 10 and 20 min and sixty min incubation showed saturation of 3-O-methyl-D-glucose transport into uterine cells. These results closely coincided with those of Roskoski Steiner (2). In order to study the relationship between phospholipid synthesis and sugar transport both being stimulated in an early period after estrogen injection, influence of phospholipid on sugar transport was investigated. Fig. 3-a shows the influence of 0, 100, 200, and 300 μgP per ml medium of phosphatidylcholine on sugar transport in the spayed
It was observed that the addition of phosphatidylcholine was effective for rate increase of intracellular 3-0-methyl-D-glucose space, the optimal dose being 100 μgP per ml medium. Fig. 3-b shows the influence of phosphatidylethanolamine. Results showed a similar tendency with regard to the effects of phosphatidylcholine. Influence of temperature on sugar transport was investigated to determine the character of this phospholipid effect. Table 1 shows that addition of phosphatidylcholine (100 μgP) to the incubation medium increased the rate of intracellular 3-0-methyl-D-glucose space to about 30% of control when incubation was done at 37°C. On the other hand, when incubation was carried out at 4°C, intracellular 3-0-methyl-D-glucose space in both addition and non-addition of phosphatidylcholine was not observed. Table 2 shows the effect of lipid on sugar transport in rat uterus. Animals used in experiment 1 and 2 were ovariectomized adult rats weighing approx. 100 g. In experiment 3 and 4 ovariectomized immature rats weighing 40-50 g were used. On sugar transport, addition of phosphatidylethanolamine, phos-

![Fig. 3. Influence of phospholipids on sugar transport in spayed rat uterus.](image)

Each point represents the mean of 4-5 rats and the vertical line through point represents standard errors of the mean. Results were shown as intracellular space.

<p>| Table 1. Influence of temperature on the sugar transport in the spayed rat uterus. |</p>
<table>
<thead>
<tr>
<th>Condition</th>
<th>Sorbitol space</th>
<th>Glucose space</th>
<th>Intracellular space</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>227 ± 11.3</td>
<td>387 ± 12.4</td>
<td>160 ± 16.8</td>
<td>+30</td>
</tr>
<tr>
<td>Ph-Ch added</td>
<td>209 ± 5.5</td>
<td>418 ± 13.0</td>
<td>209 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>217 ± 9.3</td>
<td>209 ± 7.6</td>
<td>-8</td>
<td></td>
</tr>
<tr>
<td>Ph-Ch added</td>
<td>185 ± 6.3</td>
<td>184 ± 7.3</td>
<td>-1</td>
<td></td>
</tr>
</tbody>
</table>

Tabulated values (μl/g) are averages of 4-5 rats ± standard error.
Fundamental condition is described under Methods.
Phosphatidylcholine (Ph-Ch) was added to 100 μgP per ml of incubation medium.
Intracellular space is calculated as the difference between the 3-0-methylglucose space and sorbitol space in the uterus.
TABLE 2. Effect in vitro of lipid on sugar transport in rat uterus.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Control</th>
<th>Intracellular space (µl/g)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>Ph-Ch</td>
<td>169 ± 19.84 (4)</td>
<td>± 36</td>
</tr>
<tr>
<td>2*</td>
<td>Ph-ET</td>
<td>230 ± 11.14 (4)</td>
<td>± 25</td>
</tr>
<tr>
<td>3**</td>
<td>Control</td>
<td>165 ± 12.87 (4)</td>
<td>± 25</td>
</tr>
<tr>
<td>4**</td>
<td>Ph-Ch</td>
<td>207 ± 6.27 (4)</td>
<td>± 25</td>
</tr>
<tr>
<td></td>
<td>Ph-ET</td>
<td>46 ± 7.71 (4)</td>
<td>± 43</td>
</tr>
<tr>
<td></td>
<td>Ph-Ino</td>
<td>66 ± 5.55 (4)</td>
<td>± 50</td>
</tr>
<tr>
<td></td>
<td>Choles.</td>
<td>69 ± 4.94 (4)</td>
<td>± 50</td>
</tr>
<tr>
<td></td>
<td>S.A.</td>
<td>33 ± 3.96 (5)</td>
<td>± 50</td>
</tr>
</tbody>
</table>

* Ovariectomized adult rat uterus.

** Ovariectomized immature rat uterus.

Results are given as the average (µl/g) ± standard error, with the number of animal in parentheses.

Phosphatidylethanolamine (Ph-Et), phosphatidylcholine (Ph-Ch) and phosphatidylinositol (Ph-Ino) were added to 100 µgP per ml medium. Two µmoles of cholesterol (Choles.) and 5 µmoles of stearic acid (S.A.) were added to incubation medium.

Experiments were carried out as described under Methods.

Fig. 4 presents a comparison of phosphatidylethanolamine effects on sugar transport between the untreated and estrogen-treated (4 hr) spayed uterus of rats. The effect of phosphatidylethanolamine for spayed rat uterus was observed to be an increase of about 35% on the rate of intracellular 3-0-methyl-D-glucose uptake. The estrogen-treated group whose phospholipid increased in the early phase by estrogen injection showed poor effects on sugar transport.

** DISCUSSION **

Results of this study indicate the phospholipid increase transport of non-metabolized
sugar, 3-0-methyl-(14C)-D-glucose, in vitro in spayed rat uterus, while estrogen-treated uterus in which phospholipids are increasing showed little effect. Estrogen injection to spayed rats rapidly enhanced the phospholipid synthesis and it was found that 3-0-methylglucose transport in vivo and in vitro into uterus increased in the early phase of hormone administration (1, 2, 12). It is an established fact that phospholipid in tissues is an important component of membrane which is composed mainly of lipids and protein, even though the basic arrangements of these two components are not yet clear. Mechanism of the phospholipid effect in Fig. 3 is still obscure but three points can be presumed in regard to effects on sugar transport: 1) Stimulation of the enzymic system related to transport as this was not observed at 4°C (Table 1), 2) Enhancement on carrier activity, in membrane, 3) Influence on conformational change in cell membrane. On the other hand, cholesterol and stearic acid related to membrane component were not observed to be related to the increase on the rate of intracellular sugar uptake. These are reports which discussed carrier activity and phospholipid on permeability (13-15). Recent studies on molecular interactions in biological membrane using NMR spectroscopy, reported by Chapman et al. (16), showed that the N+(CH3)2 protons of phosphatidylcholine in biological membrane are in a similar environment to that observed with lecithin alone in 2H2O and have freedom of molecular motion. As show in Fig. 4 phospholipid effect on sugar transport was hardly recognizable in the estrogen treated uterus, thus, acceleration by estrogen of the early phospholipid metabolism may correlate to sugar transport. Accordingly, it can be concluded that phospholipid takes part in the mechanism of estrogen action.

Recently, effects of phospholipases on sugar and amino acid transport in the rat uterus were investigated in this laboratory. Detailed reports are now being prepared.

SUMMARY

Role of phospholipid on the sugar transport in the rat uterus was examined by the use of non-metabolizable sugar 3-0-(14C)methyl-D-glucose and D-(1-3H) sorbitol. Results are summarized as follows:

1. Rate of 3-0-methyl-D-glucose uptake was increased by estrogen injection, but the rate of D-sorbitol uptake into rat uterus was changed little after hormone treatment.

2. Maximal response of estrogen effect (4 hr) was observed by an incubation time of 10 to 20 min.

3. Addition of phosphatidylcholine or phosphatidylethanolamine in incubation medium in the spayed rat uterus was effective on sugar transport for increase of the rate of intracellular 3-0-methyl-D-glucose space. The estrogen-treated group showes a poor effect.

4. When incubation was carried out at 4°C, the intracellular 3-0-methyl-D-glucose space was not observed with either addition and non-addition of phospholipid.

5. Rate of intracellular 3-0-methyl-D-glucose space was not influenced by the addition of cholesterol and stearic acid in the incubation medium.
REFERENCES

12) SPAZIANI, E. AND SUDDICK, R.P.: Endocrinol. 81, 205 (1967)