THE ACTIVATING EFFECT OF PHOSPHOLIPIDS ON THE ATP-ASE ACTIVITY AND Ca^{++} TRANSPORT OF FRAGMENTED SARCOPLASMIC RETICULUM

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The inhibition by phospholipase C of the ATP-ase activity (Kielley and Meyerhof, 1950) and relaxing effect (Ebashi, 1957) of skeletal muscle microsomes and the parallelism between the inhibition of the ATP-ase activity and the disappearance of lecithin (Kielley and Meyerhof, 1950) have long suggested the involvement of phospholipids in these processes. It has also been shown that the inhibition of the ATP-ase activity is not explained by the accumulation of diglycerides and phosphorylcholin, the products of the reaction of phospholipase C with lecithin (Kielley and Meyerhof, 1950).

Treatment of microsomes with phospholipase C leads also to the inhibition of the Ca uptake, which occurs parallel with the inhibition of the ATP-ase activity and is accompanied by a decrease in the lecithin content of the microsomes (Table 1.)

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Preparation	ATP-ase activity μ moles of Pi per mg protein per minute	Ca uptake μmoles of Ca per mg protein	Phospholipid content μmoles of P per mg protein	
			lecithin	other phospholipids
Untreated microsomes	2.5	1.4	0.18	0.10
Phospholipase C treated microsomes	0.22	0.10	0.028	0.084

Table 1. The effect of phospholipase C on the ATP-ase activity and Ca uptake of skeletal muscle microsomes. Phospholipids were determined after extraction according to Folch et al. (1957) by the method of Marinetti et al. (1957). Ca uptake was measured at a final protein concentration of 0.02 mg per ml. For other details see legend to Fig. 1.

No changes in the concentration of other phospholipids were found. Significantly, the disappearance of lecithin from phospholipase C treated microsomes was not accompanied by the formation of lysolecithin as shown by the constancy of the amount of non-lecithin phospholipids before and after treatment with phospholipase C. On this basis the liberation of free fatty acids can also be excluded.

The microsomal structure which is required for the ATP-ase activity and the transfer of Ca does not undergo irreversible change after the removal of about 90% of the microsomal lecithin since addition of lecithin or lysolecithin to the microsomes treated with phospholipase C causes full recovery of both Ca^{++} transport and ATP-ase activity (Fig. 1 and Table 2). Lysolecithin produced 5-10 fold activation of the ATP-ase and Ca^{++} transport when added in amounts as low as 0.07 μmoles of lysolecithin per mg microsomal protein. The optimum concentration for the activation of ATP-ase and Ca^{++} transport by lysolecithin was about 0.03-0.05 mg per ml (Fig. 1).

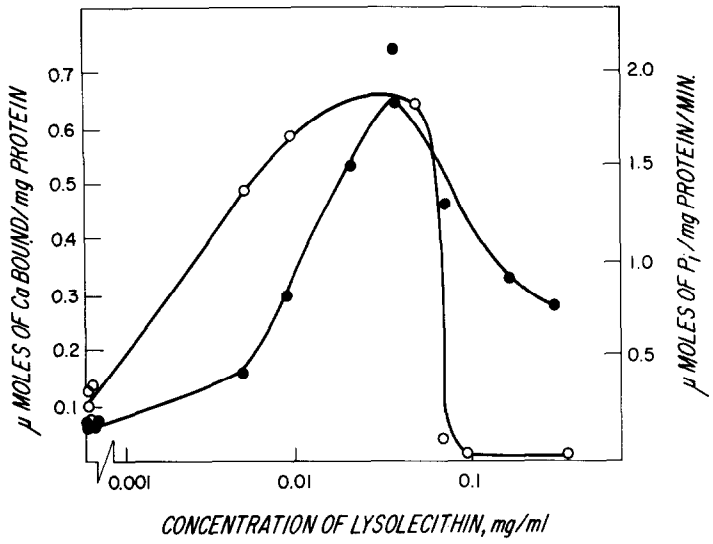

Fig. 1. Effect of lysolecithin on the Ca^{++} uptake and ATP-ase activity of sarcoplasmic reticulum fragments. Rabbit skeletal muscle microsomes prepared as described by Nagai et al. (1960) were treated with 1 mg of phospholipase C per ml in a solution containing 0.1M KCl, 5 mM histidine, pH 7.3, 0.5 mM $CaCl_2$ and 8.6 mg microsomal protein per ml for 30 minutes at room temperature. The microsomes were removed by centrifugation at 80,000 g for 30 minutes. The sediment was resuspended in 0.1M KCl, 5 mM histidine solution at pH 7.5 and the centrifugation repeated. The sediment was resuspended in 0.1M KCl, 5 mM histidine, pH 7.3, final concentration of protein, 3 mg per ml. The Ca^{++} uptake was measured in a medium containing 0.1M KCl, 5 mM histidine, 5 mM oxalate, 5 mM $MgCl_2$, 5 mM ATP, 0.1 mM $Ca^{45}Cl_2$ and 0.15 mg protein per ml. Total volume 2.0 ml, pH 7.3. The reaction was started with ATP 2 minutes after the addition of microsomes to the phospholipid containing reaction mixture. After 15 minutes incubation at room temperature, the microsomes were removed by filtration through Millipore filter (Type HA with 0.45 micron average pore diameter) and the radioactivity of the protein free filtrate was determined by the method of Loftfield and Eigner (1960). The amount of bound Ca^{++} was calculated from the difference between total radioactivity and the radioactivity of the microsome free filtrate. The Ca^{++} uptake of control microsomes was 0.66 μmole of Ca per mg of protein in the absence of phospholipids.

ATPase activity was measured in a medium containing 10 mM Tris buffer, pH 7.3, 5 mM $MgCl_2$, 5 mM ATP, and 0.075 mg protein per ml. Total volume 2 ml. Temperature 33°. ATPase assay was started by adding ATP two minutes after the addition of microsomes to the phospholipid containing reaction mixture. Reaction was stopped after five minutes by the addition of 0.5 ml of 10% trichloroacetic acid. Two ml aliquots of the protein free solution were used for the determination of inorganic phosphate by the method of Fiske and Subbarow (1925). Control microsomes liberated 1.2 μmole Pi per mg protein per minute in the absence of phospholipids.

Symbols: ● - ● ATP-ase activity; O - O Ca^{++} uptake.

Qualitatively similar effects were obtained with other phospholipids, however, the concentration range at which maximum activation occurred was usually higher (0.1-0.5 mg/ml) and less defined than in the case of lysolecithin (Table 2).

Added Phospholipid	Final concentration mg/ml	ATP-ase activity µmoles of Pi per mg protein per minute	Ca uptake µmoles of Ca^{++} per mg protein
None	-	0.15	0.12
dl-α-dipalmitoyl-lecithin	0.1	1.20	0.59
calf brain lecithin	0.1	1.20	0.50
Egg lecithin	0.5	0.6*	0.6
soy lecithin	0.5	0.25*	0.12*
l-α-dipalmitoyl-lecithin	0.2	1.05	0.60
Phosphatidyl-serine	0.3	0.35	0.25
dl-α-cephalin	0.5	0.15	0.20

Table 2. Effect of various phospholipids on the ATP-ase activity and Ca^{++} uptake of phospholipase C treated microsomes.

The Ca^{++} uptake and ATP-ase activity were measured as described in the legend to Fig. 1. The data represent maximum activities at optimum activating concentration of phospholipids with the exception of those labelled with asterisk where no optimum concentration could be established.

Phosphatidylserine and dl-α-cephalin were obtained from Mann Research Laboratories. All other phospholipids and phospholipase C were purchased from Nutritional Biochemicals, Inc. The purity of the phospholipids was tested by paper chromatography according to Marinetti et al. (1957). Lysolecithin, l-α-dipalmitoyl-lecithin, egg lecithin, phosphatidylserine, and dl-α-cephalin were chromatographically homogeneous. DL-α-dipalmitoyl-lecithin and calf brain lecithin contained about 90 and 82% lecithin respectively, the contaminant being lysolecithin.

In optimum concentrations dl-α-lecithin, l-β-lecithin, calf brain lecithin and egg lecithin were about as effective as lysolecithin in restoring the ATP-ase activity and Ca^{++} uptake of phospholipase C treated microsomes. The least effective of the preparations tested were soy lecithin, phosphatidylserine and dl-α-cephalin. The concentrations of phospholipids required for the activation of ATP-ase and Ca^{++} uptake are similar, in accordance with the suggested correlation between these processes (Hasselbach and Makinose, 1961).

The phospholipid concentration for optimum activation of both ATP-ase activity and Ca^{++} uptake increases with increasing concentration of microsomes. A tight binding of added phospholipids to microsomes is indicated by the fact that lysolecithin or dl-α-lecithin applied in optimum concentration is not removed from the microsomes by repeated washing with 0.1M KCl and 5 mM histidine, pH 7.3. This was ascertained by chromatographic analysis of lysolecithin treated microsomes and by the fact that the activation by lysolecithin of the ATP-ase activity is not lost during washing.

Addition of phospholipids to untreated microsomes caused an about 2 fold activation of ATP-ase activity while no activation of the Ca^{++} uptake could be detected.

The absence of any phosphate exchange in lecithin during ATP hydrolysis and Ca uptake using ATP^{32} of high specific activity tends to exclude the possibility of direct participation of lecithin in the transfer of Ca^{++} in a manner suggested by Hokin and Hokin (1961) for the role of phosphatidic acid in the Na:K transport.

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