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The binding of ouabain to Na^+–K^+ -dependent ATPase treated with phospholipase

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SUMMARY

1. An (Na^+–K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) preparation treated with phospholipase showed nearly the same kinetics of [^{3}H]ouabain binding as those of the control.

2. Phospholipase-treated ATPase preparation which bound ouabain showed no activation of (Na^+–K^+)-ATPase activity in the presence of phospholipids.

3. The experiments suggested that the binding of ouabain to (Na^+–K^+)-ATPase did not require phospholipids directly.

When an Na^+–K^+-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) preparation from ox brain microsomes was treated with phospholipase A (phosphatide acylhydrolase, EC 3.1.1.4), its ATPase activity was reduced to 20–25% of the control value and approximately 70% of phospholipids in the preparation were changed to lyso-compounds. When the preparation was treated with phospholipase C (phosphatidylcholine cholinephosphohydrolase, EC 3.1.4.3), both the ATPase activity and the content of phospholipids of the preparation were reduced to 40% of the control value, respectively. After the preparation had been treated with phospholipase A or C, its ATPase activity was reactivated to nearly the control value by addition of acidic phospholipids. Treatment with phospholipase A reduced the rate of phosphorylated enzyme formation by about 60% and reduced the rate of phosphorylated enzyme decomposition by more than 90%. The rates of formation and decomposition of the phosphorylated enzyme were increased to the control level and to 40% of the control level,respectively, by addition of acidic phospholipids. From these results, Taniguchi and Tonomura[1] have proposed that the role of phospholipids in (Na^+–K^+)-ATPase is to give a negative charge and hydrophobicity in the vicinity of the active site of ATPase, rather than as components of the active site itself.

Abbreviation: GEDTA, glycoletherdiaminetetraacetic acid.

In this report, the binding of ouabain to (Na^+-K^+)-ATPase treated with phospholipase will be described.

The method of partial purification of (Na^+-K^+)-ATPase was reported previously[1]. The ATPase preparation was treated with phospholipase A prepared from *Naja naja* venom according to Imai and Sato[2] or phospholipase C (*Clostridium wellchii*) in incubation mixture containing 1 mg/ml ATPase protein, 0.1 mg/ml phospholipase A or 0.4 mg/ml phospholipase C, 2.95 M sodium acetate or 1.1 M sucrose, 29.5 mM Tris, 67.5 mM 2-mercaptoethanol, 0.07 mM EDTA and 11.2 mM $CaCl_2$ (pH 7.4) at 37°. The treatment was terminated by addition of GEDTA (glycoletherdiaminetetraacetic acid)–Tris–HCl buffer (pH 7.4) at a final concentration of 28.6 mM GEDTA and 190 mM Tris.

Binding of [^{3}H]ouabain to the ATPase was measured in 1 ml of reaction medium by the method of Barnett[3] with slight modifications. The ATPase preparation (0.067 mg/ml) was preincubated with 5 mM $MgCl_2$, 140 mM NaCl, 196 mM sodium acetate, 14 mM KCl, 3 mM ATP, 32 mM sucrose, 24.6 mM Tris, 4.5 mM 2-mercaptoethanol, 1.91 mM GEDTA and 0.745 mM $CaCl_2$ (pH 7.4) at 37° for 5 min. The reaction was started by addition of [^{3}H]ouabain ($4 \cdot 10^8$ counts/min per μmole) at the final concentration of 1.75 μM. At various time intervals, cold unlabeled ouabain (pH 7.4 with 40 mM Tris–HCl) was added to the reaction medium at the final concentration of 5 mM to stop the reaction. The enzyme suspension was immediately filtered through a membrane filter (pore size 0.45 μm) and washed with 100 ml of cold 100 μM ouabain (pH 7.4) and 75 ml of cold water. The ^{3}H present on the filter was counted after dissolving in 12 ml of Bray's[4] solution.

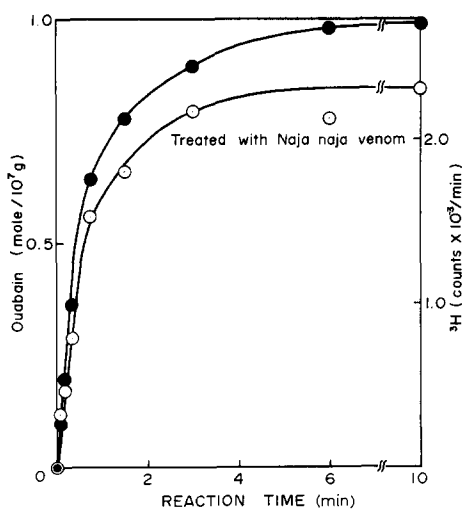

Fig. 1. Ouabain binding to (Na^+-K^+)-ATPase. Amount of ouabain bound to 10^7 g protein; ○–○, phospholipase A-treated for 15 min; ●–●, control preparation.

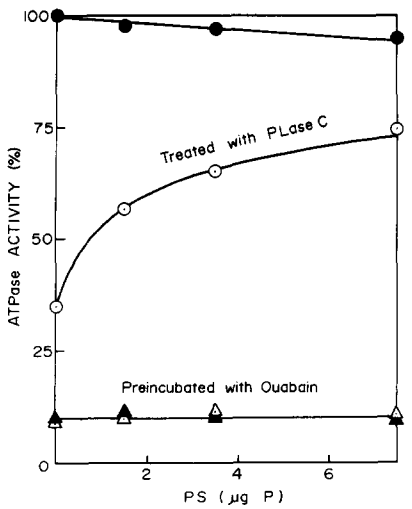

Fig. 2. Reactivation of (Na^+-K^+)-ATPase activity by phosphatidylserine (PS) in the presence or absence of ouabain. ATPase preparation (0.1 mg/ml) treated with phospholipase C for 2 h was incubated with 5 mM $MgCl_2$, 140 mM NaCl, 3 mM ATP, 32 mM sucrose and 0.5 μM ouabain (pH 7.4) at 37° for 10 min. ATPase activity was measured 10 μg/ml ATPase protein, 5 mM $MgCl_2$, 140 mM NaCl, 14 mM KCl and various concentrations of phosphatidylserine in the absence or presence of 0.05 μM ouabain. ○–○, △–△, phospholipase C-treated; ●–●, ▲–▲, control; △–△, ▲–▲, preincubated with ouabain.

The measurement of ATPase activity, the estimation of protein concentration and other details were similar to those reported[1].

The time course of [^{3}H]ouabain binding to (Na^+–K^+)-ATPase preparation was shown in Fig. 1. The final amount bound was approximately 1 mole/10^7 g protein for the control and 0.85 mole/10^7 g protein for phospholipase A-treated preparation and 0.80 mole/10^7 g protein for phospholipase C-treated preparation (data not cited here). The time required for half the maximum binding was approximately 30 sec for those preparations, while the ATPase preparation treated with phospholipase A (data not cited here) or C (Fig. 2) showed a marked decrease in (Na^+–K^+)-ATPase activity as reported previously[1].

ATPase preparations treated with ouabain showed no activation of the ATPase activity in the presence of phosphatidylserine as shown in Fig. 2, while phospholipase C-treated ATPase preparation showed a marked activation of the (Na^+–K^+)-ATPase activity in the presence of phosphatidylserine.

Approximately 70% of phospholipids in the preparation were changed to lyso-compounds by treatment with phospholipase A, while the content of phospholipids was reduced to approximately 40% by treatment with phospholipase C[1]. It was interesting that ouabain did bind (Na^+–K^+)-ATPase in spite of phospholipase A or C treatment.

These facts strongly supported that the binding of ouabain to (Na^+–K^+)-ATPase does not require phospholipids directly and that the role of phospholipids in (Na^+–K^+)-ATPase is to give a negative charge and hydrophobicity in the vicinity of the active site of ATPase, rather than as components of the active site itself.

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