Reversal by Lecithin of the Amobarbital-Induced Inhibition of Microsomal (Na + K)-Activated ATPase

Enzymatic activity of (Na+K)-ATPase is dependent to a large extent on the presence of associated phospholipids as evidenced by the marked fall in activity induced by phospholipase treatment of membrane preparations containing the enzyme[1]. Phospholipids are known to activate (Na+K)-ATPase from different sources[2] although the exact mechanism involved is still speculative. Membrane-active drugs often involve interactions with phospholipids which constitute a sizable portion of the membrane structure. In the case of the myocardium, these interactions might be expected to modify transmembrane ionic fluxes which are important determinants of contractility[3]. In the present paper we report the effect of sodium amobarbital (amytal), a drug known to induce depression of myocardial contractility[4] on (Na+K)-ATPase of cardiac sarcoplasmic reticulum.

Sarcoplasmic reticulum was prepared from the left ventricular wall of adult mongrel dogs. The tissue was homogenized in a solution containing 0.3 *M* sucrose, 20 m*M* *tris*-HCl (pH 7.6), 1 m*M* EDTA and 5 m*M* sodium azide. The homogenate was centrifuged at 1.000 $\times g$ for 20 min to separate the cellular debris. The supernatant was subsequently spun at 100.000 $\times g$ for 90 min to obtain the microsomal fraction. The final pellet was suspended in the homogenizing medium to a protein concentration of 1 mg/ml.

(Na+K)-ATPase activity was measured in terms of inorganic P liberated from ATP by the enzyme. The assay medium contained 5 m*M* $MgCl_2$, 100 m*M* NaCl, 10 m*M* KCl, 25 m*M* *tris*-HCl (pH 7.6) and the membrane preparation (100 ug protein/ml). The drug was added as 0.1 ml of either 2 m*M*, 4 m*M* or 6 m*M* solution. The effect of lecithin was determined by adding 0.5 mg of purified egg lecithin (purchased from Sigma Co.). For the purpose of this study lecithin was suspended in a solution containing 0.3 *M* sucrose, 1m*M* EDTA and 10 m*M* *tris*-HCl (pH 7.4) and 'solubilized' by sonication. (Na+K)-ATPase was also determined in the presence of 0.5 mg lecithin preincubated for 10 min with 4 m*M* or 6 m*M* sodium amytal. The ATPase reaction was started in all instances by the addition of the microsomal membrane preparation and was carried out for 10 min. At the end of this incubation period 10% trichloroacetic acid was added and the inorganic P liberated was measured by the Fiske-Subbarow method[5]. The ATPase activity in the absence of Na^+ and K^+ (Mg^{++}-ATPase) was also determined and subtracted from the total ATPase.

As shown in Table I, sodium amytal significantly inhibited (Na+K)-ATPase of dog heart microsomes. This inhibition was not complete under the experimental conditions used. It was practically completely reversed by the subsequent addition of lecithin and, in this respect, resembled the inhibition of mitochondrial ATPase by oligomycin[6]. In contrast, in a separate series of experiments not detailed here, ouabain-induced inhibition of (Na+K)-ATPase was not reversed by lecithin even in high concentrations. The mechanism of inhibition of the enzyme by the two substances would appear, therefore, to be different; the functional implications of this inhibition may also differ. Amytal has been shown to bind to membrane phospholipids[7] or phospholipid bilayers[8] and this binding may be an essential step in the (Na+K)-ATPase inhibition in view of the requirement for phospholipids for ATPase activity. That this may be so is also indicated by the effects of lecithin pre-incubated with sodium amytal. As seen in Table II, (Na+K)-ATPase was significantly inhibited under these conditions. This is an indication that the lecithin-amytal association is readily reversible since the complex can confer the inhibitory properties of the drug. The exact functional implications of this phospholipid-amytal interaction in cardiac microsomes in particular as regards the reported[4] inhibition of microsomal Ca^{++} uptake by amytal, is not clear at present.

Table I.

Additions	(Na+K)-ATPase activity (μmol P_i/mg protein/10 min)
None	12.9 ± 0.6
2 m*M* sodium amytal	10.3 ± 0.3
4 m*M* sodium amytal	5.8 ± 0.7
6 m*M* sodium amytal	2.4 ± 0.9
2 m*M* sodium amytal + 0.5 mg lecithin	13.1 ± 0.7
4 m*M* sodium amytal + 0.5 mg lecithin	10.9 ± 0.2
6 m*M* sodium amytal + 0.5 mg lecithin	11.2 ± 0.4

Table II.

Additions	(Na + K)-ATPase activity (μmol P_i/mg protein/10 min)
None	11.4 ± 0.2
0.5 mg lecithin + 4 m*M* amytal[a]	6.1 ± 0.1
0.5 mg lecithin + 6 m*M* amytal[a]	3.4 ± 0.2

[a] Pre-incubated for 10 min

Résumé. L'amobarbital de soude inhibe l'activité du (Na+K)-ATPase du réticule sarcoplasmique isolé. Cette inhibition peut être évitée par l'addition de lécithine purifiée. La lécithine pré-incubée en présence de l'amobarbital inhibe (Na+K)-ATPase.

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