KOREAN JOURNAL OF ZOOLOGY
Vol. 20, No.2, 101~107 (1977)

Studies on the ATPase Activity and Calcium Transport of Fragmented Sarcoplasmic Reticulum

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膜 ATPase活性과 Ca 透過性에 관한 硏究

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(Received May 12, 1977)

摘 要

토끼의 골격근 小胞體의 ATPase活性과 Ca輸送에 대한 sodium azide, cAMP, G-strophanthin 및 dicumarol의 영향을 측정하였다.

Sodium azide(0.05mM)와 G-strophanthin(0.25mM)은 ATPase活性과 Ca輸送能에 아무런 영향도 미치지 아니하였다. cAMP(1×10^{-6}M~5×10^{-4} M)는 ATPase活性에는 아무런 영향도 미치지 않았으나 Ca輸送은 억제하였다. Dicumarol(0.05mM)도 ATPase 活性에는 영향이 없었으나 小胞體의 8,000~12,000×G分劃에서의 Ca輸送을 억제하였다.

INTRODUCTION

The ATPase activity of the fragmented sarcoplasmic reticulum of skeletal muscle has been known to be closely linked to the active transport of calcium in the reticulum, and various biochemical properties of the enzyme activity and its relation to the Ca uptake have widely been studied. A number of ions, metabolic inhibitors, and pharmaceutical drugs have also been studied for their effects on the ATPase activity.

Most of these studies, however, were done on the enzyme activity or on the Ca uptake separately and hence any result obtained on the ATPase activity may

本硏究는 財團法人 産學協同財團의 1976年度 學術硏究費 支援을 받아 이루어진 것임.

not be directly applicable to the function of Ca uptake.

Sodium azide is known to inhibit the Ca uptake and the ATPase activity in mitcchondria (Fanburg and Gergely, 1965). cAMP has been known to be involved in the Ca transport of living membranes (Rasmussen, 1970) soon after the findings of Rabinowitz (1965) that adenyl cyclase is distributed in the rabbit skeletal muscle and of Entman (1969) that the same enzyme is also distributed in the dog cardiac sarcoplasmic reticulum. G-strophanthin is well known to inhibit the $(Na^+ + K^+)$—ATPase (Charnock and Potter, 1969). Dicumarol is known to inhibit the Ca uptake of the mitochondrion when the uptake is supported by the oxidative phosphorylation (Weber 1968).

In the present paper, the effects of sodium azide, adenosine 3′, 5′-cyclic monophosphate (cAMP), G-strophanthin, and dicumarol on the ATPase activity and Ca uptake were studied and the effects were compared with respect to the enzymatic activity and Ca transport.

MATERIALS AND METHODS

Fragmented scacoplasmic reticulum was prepared by the method described elsewhere (Ha, 1971 and 1972). The fractions sedimented between 8,000—12,000, 12,000—20,000 and 20,000—36,000 xG were used throughout the experiments. These fractions are hereafter referred to as H, M and L fractions, respectively. Preparations not older than 72 hours were used. Protein content was measured by Lowry's method (Lowry, 1951).

ATPase activity and Ca uptake were determined with essentially the same methods as described elsewhere (Ha, 1972). Reaction mixture for determination of ATPase activity consisted of 20 mM tris-maleate buffer (pH 6.8), 50mM KCl, 4mM $MgCl_2$, 0.2 mM $CaCl_2$, 2mM ATP and test chemicals in the concentrations specified in Figures and Tables. Reaction was started by the addition of reticulum fragments (final concentration: 0.05—0.1mg protein/ml). Calcium uptake was measured in the reaction mixture consisting of 20mM tris-maleate buffer (pH 6.8), 50mM KCl, 50mM NaCl, 4mM $MgCl_2$, 0.02mM $CaCl_2$ containing ^{45}Ca in the specific activity of approximately 1×10^7 cpm/ml, 2mM ATP, and other additions as specified in the text. The reaction was initiated by the addition of reticulum fragments of 0.05—0.2mg protein/ml.

Radioactivity was measured with a liquid scintillation counter using Bray's solution (Bray, 1960) as the scintillator.

RESULTS

In the present experiment, the muscle homogenate was fractionated into three

fractions by centrifugation and each fraction was measured for the ATPase activity and Ca uptake. The three fractions were different in their ATPase activity and in the amount of Ca they took up.

As seen in Fig. 1, the reaction rate of the ATPase expressed in μmoles Pi/mg protein/min. was the highest in the L fraction followed by the M fraction. The H fraction had the lowest enzymatic activity. The reaction rates of the three fractions decreased lineally with time between 2—20 minutes.

The capacity of Ca uptake was the highest in the H fraction and the L fraction was the least. The rate of Ca uptake of all the three fractions was very rapid and the maximum uptake was observed within one minute. The accumulated Ca was slowly released into the medium after one minute of incubation, as observed previously (Ha, 1972).

The addition of sodium azide in the concentration of 0.05mM to the H, M and L fractions did not change the ATPase activity suggesting that there was practically no significant contribution of the mitochondrial part to the liberation of inorganic phosphate (Pi) from ATP in these fractions (Table 1).

As in the case of the ATPase activity, sodium azide caused practically no inhibition of Ca uptake of all fractions (Table 2).

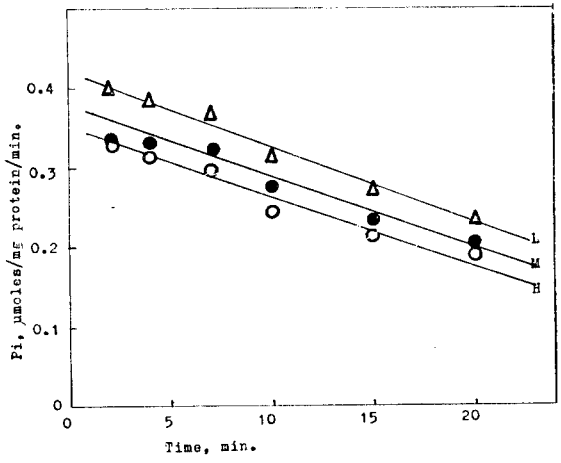

Fig.1. The reaction rate of the ATPase of the H,M and L fractions of fragmented sarcoplasmic reticulum.

The addition of G-strophanthin in the concentration of 0.25mM or 2.5mM did not cause any significant change in the ATPase activity. The ATPase activity of either H, L or M fraction was not inhibited by G-strophanthin (Table 3).

G-strophanthin did not affect the Ca uptake of either M or L fractions as was in the case of ATPase activity (Table 4).

Table 1. The effect of sodium azide on the ATPase activity of fragmented sarcoplasmic reticulum

Fraction	H	M	L
Control	0.22	0.28	0.33
NaN_3(0.05mM)	0.21	0.28	0.31

ATPase activity is expressed in μmoles Pi/mg protein/min. The reaction was carried out at 30°C for 10 minutes.

Table 2. The effect of sodium azide on the Ca uptake of fragmented sarcoplasmic reticulum

Fraction	H	M	L
Control	160.6	142.6	130.5
NaN_3 (5×10^{-5}M)	155.7	140.1	131.2

Ca uptake is expressed in μmoles Ca/g protein/min. The reaction was carried out at 30°C for 1 minute.

Table 3. The effect of G-strophanthin on the ATPase activity of fragmented sarcoplasmic reticulum

Fraction	H	M	L
Control	0.027	0.032	0.034
G-strophanthin (0.25mM)	0.28	0.33	0.35
G-strophanthin (2.5mM)	0.27	0.32	0.34

ATPase activity is expressed in μmoles Pi/mg protein/min. The reaction was carried out at 30°C for 10 minutes.

Table 4. The effect of G-strophanthin on the Ca uptake of fragmented sarcoplasmic reticulum

Fraction	M	L
Control	92.0	74.2
G-strophanthin (0.25mM)	92.8	80.1

Ca uptake is expressed in μmoles Ca/g protein/min. The reaction was carried out at 30°C for 1 minute.

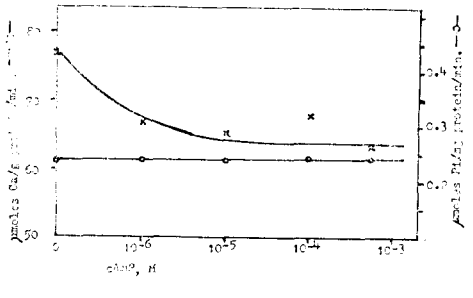

Fig. 2. The effect of cAMP on the ATPase activity and Ca uptake of the fragmented sarcoplasmic reticulum (M fraction).

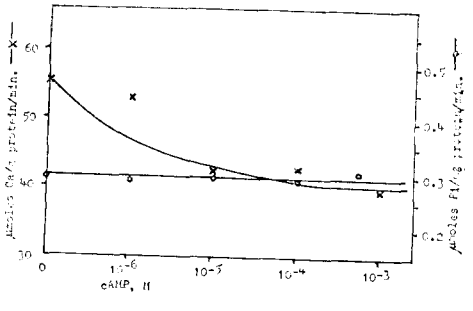

Fig. 3. The effect of cAMP on the ATPase activity and Ca uptake of the fragmented sarcoplasmic reticulum(L fraction).

The ATPase activity was not inhibited by the addition of cAMP in the concentrations between 1×10^{-6}M and 5×10^{-4} (Figs. 2 and 3). Dibutyryl cAMP (DBcAMP) in the concentration of 5×10^{-4}M either did not inhibit the enzyme activity. Unlike cAMP or DBcAMP, 1mM theophylline increased the enzyme activity of M fraction by 10 to 20%. This stimulating effect was the same whether theophylline was added alone or with cAMP to the reaction system. The L fraction, however, did not respond to the addition of theophylline.

The Ca uptake of both M and L fractions was inhibited, though to a small extent, by cAMP. As seen in Figs. 2 and 3, cAMP of 10^{-6} or 10^{-5} M caused an inhibition of Ca uptake by 10 to 20%. Higher concentrations than 10^{-5}M seemed not to increase the inhibitory effect.

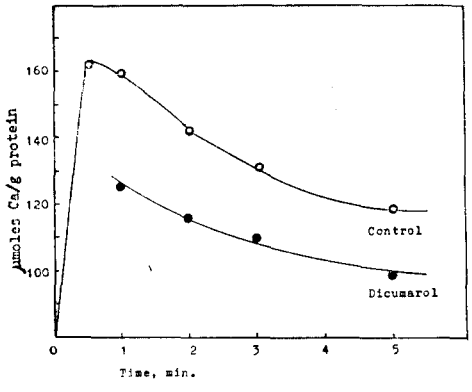

Fig. 4. The effect of dicumarol(0.05mM) on the Ca uptake of fragmented sarcoplasmic reticulum (H fraction).

The ATPase activity of M and L fractions was not inhibited by the addition of 50 μM dicumarol; the H fraction, however, showed slightly decreased activity. The effect of 50 μM dicumarol on the Ca uptake was shown in Fig. 4 for the H fraction. This compound decreased the Ca uptake of the H fraction by 20 to 30%. The effect of dicumarol was, however, not significant in the M and L fractions.

DISCUSSION

The ATPase of fragmented sarcoplasmic reticulum has been reported to consist of two separate components, Mg^{++}—ATPase and ($Mg^{++}+Ca^{++}$)—ATPase (Weber, 1966; Duggan, 1968; Ha, 1974). In the present experiment, however, these two components were not dealt with separately and data presented are for total ATPase.

It is apparent that ATPase of fragmented sarcoplasmic reticulum is different from that of mitochondria in the response to sodium azide. Furthermore, it is different from ($Na^{+}+K^{+}$)—ATPase in that it is not inhibited by G-strophanthin which is a potent inhibitor for the latter ATPase.

Calcium uptake of M and L fractions of fragmented sarcoplasmic reticulum is also different from that of mitochondria in the response to dicumarol. Dicumarol is known to inhibit the Ca uptake of mitochondria (Weber, 1968). The H fraction, however, is inhibited by this compound suggesting that there might be a considerable contamination of mitochondria. In fact, the ATPase of this fraction showed a decreased activity in the presence of dicumarol. It is therefore concluded that

fragmented sarcoplasmic reticulum sedimented at above at least 12,000 xG should be considered as pure preparation. Electron micrographs and the determination of succinic dehydrogenase activity of M and L fractions also revealed that these fractions were not contaminated with mitochondria (Ha, 1967 and 1972; Batra and Daniel, 1971).

The present preparation usually contained an intrinsic Ca of an average of 35 nmoles/mg protein as measured with an atomic absorption spectrophotometer. Essentially the same value of endogenous Ca (30 nmoles/mg) was reported by Carvalho (1968). (Chevallier and Butow (1971) have reported as much as 500 nmoles/mg). This value was always taken into consideration in the subsequent calculation of the amount of Ca taken up by the fragmented sarcoplasmic reticulum.

The ATPase activity and Ca uptake of both M and L fractions of fragmented sarcoplasmic reticulum are not equally affected by sodium azide, G-strophanthin and dicumarol. Cyclic AMP, however, acts differently on the ATPase and Ca uptake. As shown in Figs. 2 and 3, ATPase activity is not changed while Ca uptake is inhibited by about 20% by the addition of 10^{-6} or 10^{-5} M cAMP. From these observations, it may be postulated that the action site of cAMP is located on the Ca transport protein and that this rotein is different entity with ATPase protein.

SUMMARY

The effects of sodium azide, cAMP, G-strophanthin and dicumarol on the ATPase activity and Ca uptake of the fragmented sarcoplasmic reticulum of skeletal muscle were studied and the effects were compared with respect to the enzymatic activity and Ca transport.

Sodium azide (0.05mM) and G-strophanthin (0.25mM) caused no inhibition on either ATPase activity or Ca uptake. cAMP($1\times10^{-6}\sim5\times10^{-4}$M) had no effect on ATPase activity while it inhibited Ca uptake. Dicumarol (0.05 mM) did not inhibit ATPase activity but caused a decreased Ca uptake of heavier fraction (8,000 —12,000xG) of the reticulum fragments.

REFERENCES

Batra, S.C. and E.E. Daniel, 1971. ATP-dependent Ca uptake by subcellular fractions of uterine smooth muscle. *Comp. Biochem. Physiol.* **38** (A):369-386.

Bray, G. A., 1960. *Anal. Biochem.* **1**: 279-285.

Carvalho, A. P., 1968. Calcium-binding properties of sarcoplasmic reticulum as influenced by ATP, caffeine, quinine, and local anesthetics. *J. Gen. Physiol.* **52**:622-642.

Charnock, J.S. and H.A. Potter, 1969. The effect of Mg^{2+} and ouabain on the incorporation of P^{32} from γ-ATP^{32} into Na^+— and K^+—activated adenosine triphosphatase. *Arch. Biochem. Biophys.* **134**:42-47.

Chevallier, J. and R. A. Butow, 1971. Calcium binding to the sarcoplasmic reticulum of rabbit skeletal muscle. *Biochemistry* **10**:2733-2737.

Duggan, P. F., 1968. Calcium-independent adenosinetriphosphatase activity in frog muscle microsomes. *Life Sci. Biochem. Gen. Mol. Biol.* 7(24, Pt. 2):1265-1271.

Entman, M. L., G. S. Levey, and S.E. Epstein, 1969. Demonstration of adenylcyclase activity in canine cardiac sarcoplasmic reticulum. *Biochem. Biophys. Res. Comm.* **35**:728.

Fanburg, B. and J. Gergely, 1965. Studies on adenosine triphosphate supported calcium accumulation by cardiac subcellular particles. *J. Biol. Chem.* **240**:2721.

Ha, D. B., 1967. Studies on the ATPase activity, relaxing activity and calcium uptake of rabbit skeletal muscle microsomes. *Korean J. Zool.* **10**:59-66.

Ha, D. B., 1971. Studies on the calcium uptake and ATPase activity of the fragmented sarcoplasmic reticulum. *Korean J. Zool.* **14**: 43-56.

Ha, D. B., 1972. The effects of caffeine on the ATPase activity and the calcium uptake of the fragmented sarcoplasmic reticulum of rabbit skeletal muscle. *Korean J. Zool.* **15**:163-182.

Ha, D. B., 1974. Studies on the ATPases of fragmented sarcoplasmic reticulum of rabbit skeletal muscle. *Korean J. Zool.* **17**: 93-102.

Lowry, O.M., N.J. Rosebrough, A.L. Farr, and R. L. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.

Rabinowitz, M., L. Desalles, J. Meisler, and L. Lorand, 1965. Distribution of adenylcyclase activity in rabbit skeletal muscle fractions. *Biochim. Biophys. Acta* **97**:29.

Rasmussen, H., 1970. Cell communication, calcium ion, and cyclic adenosine monophosphate. *Science* **170**:406.

Weber, A., 1966. Energized calcium transport and relaxing factors. *In:* Current Topics in Bioenergetics (edited by D. R. Sanadi). Academic Press, New York.

Weber, A., 1968. The mechanism of the action of caffeine on sarcoplasmic reticulum. *J. Gen. Physiol.* **52**:760.