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Studies on the Calcium Uptake and ATPase Activity of the Fragmented Sarcoplasmic Reticulum*

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筋小胞體의 Ca 吸收能과 ATPase 活性에 관한 硏究*

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摘 要

筋小胞體의 Ca 吸收能과 ATPase活性을 各種 濃度의 K, Mg, caffeine, procaine, 및 quinine 존재하에서 측정하였다.

ATP不在下에서의 Ca吸收能은 K 또는 Mg의 농도가 증가함에 따라 低下된다. 그러나 ATP 존재하에서의 Ca 吸收能은 K의 농도에는 거의 영향을 받지 않고, Mg의 농도가 증가함에 따라 현저히 증가한다. Caffeine과 procaine은 ATP존재하의 Ca 吸收能을 阻害하지만 quinine은 阻害하지 않는다.

ATPase 活性은 K의 농도에는 영향을 받지 않으나 Mg의 존재에 의하여 증가된다. Caffeine, procaine 및 quinine은 이 活性에 거의 영향은 미치지 않는다.

INTRODUCTION

Considerable evidence has been assembled in recent years to indicate that calcium ion may be the link between excitation and contraction in skeletal muscle.

Kinetic studies of calcum ion movements in muscle by Bianchi and Shanes (1959) led to their suggestion that the sarcoplasmic reticulum may serve as the source for calcium ion involved in the coupling mechanism. They proposed that the sarcoplasmic reticulum binds calcium ions which are released by excitation and that the release of calcium ions activates the contractile mechanism. Ebashi(1961), Hasselbach and Makinose(1963), Hasselbach(1964), and Weber *et al.* (1966) have shown that ATP in the presence of Mg induces the microsomal fraction isolated from skeletal muscle to bind calcium ion avidly. These workers proposed that the accumulation of calcium ion by the relaxing factor (the microsomes) is involved in the reaction of muscle relaxation. This fraction is composed of fragmented membranes of the tubular sarcoplasmic reticulum (and presumably of

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the transverse tubular system) which ramifies extensively within the muscle cell (Franzini-Armstrong, 1964; Peachey, 1965). This extensive membranous network with a high affinity for Ca is considered adequate to maintain the Ca associated with actomyosin in muscle at rest below the level required to activate the contractile mechanism (Weber *et al.*, 1964).

The contraction-relaxation cycle evoked in a muscle cell by depolarization of the plasma membrane is thus probably regulated by a transient increase in the concentration of free calcium ion in equilibrium with the myofibrils. Stimulation of the muscle cell releases Ca from the sarcoplasmic reticulum by a mechanism not yet understood, and the Ca so released triggers contraction by interacting with the myofibrils until it is reabsorbed by the sarcoplasmic reticulum (Sandow, 1965). The state of Ca accumulated by the sarcoplasmic reticulum is still unknown, though it is of fundamental importance to know whether this Ca is accumulated by an active transport process or merely by adsorption at binding sites of the lipoprotein membranes.

Carvalho (1966) has shown that binding sites do exist in these membranes which could account for an accumulation of cations, including Ca, of about 350 μeq/g of protein at pH 7. He also investigated the interaction of Ca, Mg and K with the microsomal fraction from the skeletal muscle of the rabbit under conditions for selective uptake of Ca, and concluded that there is an interdependence of the binding of the three cations at binding sites of the fragmented reticular membranes which suggests that the cations compete for the same binding sites and that about 80% of the Ca binding induced by ATP occurs by displacing Mg and K from the binding sites of the membranes.

Calvalho and Leo (1967) have shown that in the presence of oxalate the isolated microsomal vesicles transport Ca against an activity gradient at concentrations of external Ca of the order of 10^{-7}M, but the concentration of free Ca in the vesicles estimated from the solubility product of calcium oxalate is less than 1% of the total Ca accumulated. In the absence of oxalate, the concentration of free Ca in the vesicles may be higher, but there is no good estimate of the magnitude of the value. Ha (1967) also studied the Ca accumulation of the microsomes in the presence of oxalate and concluded that oxalate increases the Ca uptake presumably by forming calcium-oxalate complex within the microsomal vesicles.

The present study was conducted to determine under various ionic environment the nature of the interaction of Ca, Mg, and K with the microsomal fraction isolated from the skeletal muscle of rabbit. Since it has been known that ATP induces the reticular membrane to bind Ca (Weber *et al.*, 1966), and that the reticular membranes bear an ATPase activity (Ebashi and Lipman, 1962), the microsomal ATPase (EC. 3.6.1.4.) activity was also measured in association with the cation-binding properties of the membranes.

Agents that modify the excitation-contraction coupling reaction in muscle and increase the force of contraction developed during the twitch may act by modifying the

cation-binding properties of the sarcoplasmic reticulum, particularly with respect to the binding of Ca which regulates the mechanical activity of the contractile components of the muscle cell (Weber *et al.*, 1963 and 1964; Isaacson and Sandow, 1967; Carvalho, 1968 a,b). Some of these agents, or potentiators (i.e., the inorganic anion, SCN^-, I^-, Br^-, and NO_3^-), have been tested and were shown to interfere with the binding of Ca by isolated reticulum (Ebashi, 1965; Carvalho, 1968 a). Other potentiators, e.g., caffeine and quinine increase the fluxes of Ca^{45} in the muscle cell in association with their effect on contraction (Sandow, 1965; Isaacson and Sandow, 1967), which suggests that they mobilize Ca bound by the sarcoplasmic reticulum. It is reported that the alkaloid quinine (and its optical isomer quinidine) causes a potentiation of twitch tension of skeletal muscle at low concentrations ($\sim 10^{-6}$M) and contracture at high concentrations($\sim 10^{-3}$M) by Isaacson and Sandow (1967). Thus, there is a growing body of evidence suggesting that drugs which alter the contractile states of muscle do so through their ability to act on the intracellular Ca stores of the muscle, in particular those of the sarcoplasmic reticulum. This is further supported by tracer studies carried out with living frog muscles which show that these agents do indeed cause the release of intracellular Ca (Isaacson and Sandow, 1967; Carvalho and Leo, 1967).

The present study was also conducted to investigate of the effects of caffeine, quinine and of the local anesthetics, procaine, on the binding of Ca by the microsomal fraction isolated from the rabbit skeletal muscle. The microsomal enzyme activity, the ATPase activity, was simultaneously measured since the inhibition of the Ca transport by these drugs was thought to cause the inhibition of the enzyme activity.

METHODS

Fragmented sarcoplasmic reticulum (also referred to as microsomes or reticular membranes) was isolated from the microsomal fraction of the rabbit skeletal muscle by differential centrifugation according to the methods of Ebashi and Yamanouchi (1964) with some modifications. In brief, skeletal muscle was taken out from an adult rabbit and fatty materials and nervous fibers were carefully removed. The muscle was then homogenized in a four-volume of 0.01 N NaOH solution with a Waring blender and the pH during the homogenation was kept at 6.8. The homogenate was centrifuged at 2,000 $\times G$ for 20 minutes and the supernatant was again centrifuged at 12,000 $\times G$ for another 20 minutes to remove the mitochondrial fraction. The supernatant was then further centrifuged at 23,000 $\times G$ for 60 minutes and the precipitate was suspended in 0.02 M tris(hydroxymethyl)aminomethane-maleate buffer containing 0.05 M KCl. The suspension was again centrifuged at 23,000 $\times G$ for 60 minutes, resuspended in the tris buffer and kept in cold until used. Microsomes not older than 72 hours were used for the following measurements. Previous study (Ha, 1967) showed no appreciable contamination of mitochondria in the preparation thus made. The protein concentration in the suspension was usually made to about 6 mg/ml of suspension. All pro-

cedures were carried out at below 4°C. The protein concentration was determined by the biuret photometry standardized by the micro-Kjeldahl analysis of nitrogen using the rabbit serum albumin as the standard protein source.

Microsomes were incubated in the medium which consisted of basically 20 mM tris-(hydroxymethyl)aminomethane, 20 mM maleate, 20 mM NaOH to make the pH of the buffer 6.8, 50 mM KCl, and 0.2 mM $CaCl_2$ containing Ca^{45} in the specific activity of about 5,000 cpm/ml medium. This solution is hereafter referred to as "basic medium". To this basic medium were added various test substances in the concentrations as indicated in the legend of appropriate Figures and Tables. Incubation was usually done at 25°C in a metabolic shaker for 1 minute for Ca uptake experiments and for 20 minutes for the ATPase study. The incubation was started by adding prewarmed microsomes to the incubation medium. The amount of the microsomes in the reaction mixture was adjusted so as to give a microsomal protein concentration in the range between 0.45 and 1.18 mg/ml.

The Ca uptake was measured by the Millipore filtration method (Martonosi and Feretos, 1964a). Millipore filters of 0.45μ pore diameter were used throughout. Incubation mixtures containing Ca^{45} were filtered after 1 minute of incubation by vacuum suction and the filter and the filtrate were counted for radioactivity using a thin-window, gas-flow type Geiger counter. The filtration was complete within 2—3 seconds of the suction. The amount of Ca accumulated in the microsomes was calculated from the specific activities of the incubation mixture, the filter and the filtrate, and expressed as mμmoles of Ca per mg of the microsomal protein. Blank filtration was done by filtering incubation medium containing no microsomes and the blank counting was subtracted from every filter countings.

The ATPase activity of the microsomes was measured by stopping the reactiou by the addition of ice-cold trichloroacetic acid in the final concentration of 6%. The reaction mixture was then filtered on a Toyo No. 2 filter paper to remove the denatured microsomal aggregation and the inorganic phosphate contained in the filtrate was quantiatively determined by the method of Fiske-SubbaRow (1925) or by the method of Allen (1940) modified by Nakamura (1950). Both methods gave essentially the same value. ATPase activity was expressed as mμmoles of inorganic phosphate (Pi) produced per mg of microsomal protein per minute.

All solutions were prepared with glass-distilled, deionized water. The ATP was obtained from Sigma Chemical Company in the disodium form. Contamination of Ca in ATP was not removed. Other reagents were of analytical grade of Wako products (Wako Chemical Industries Co., Japan). Radioactive Ca^{45} was a product of Commissariat a L'Energie Atomique, France, and purchased through Atomic Energy Company, Seoul, Korea. Millipore filters were obtained from Millipore Corporation, U.S.A.

RESULTS

1. The rate of the Ca uptake by the microsomes.

The Ca uptake by the fragmented sarcoplasmic reticulum was measured at different time intervals of incubation and the results are shown in Fig. 1. The uptake is very rapid during the first 10 or 20 seconds of the incubation and nearly 80% of the totally accumulated Ca are taken up in this early period. In the following experiments, therefore, the Ca uptake was measured on microsomes incubated for 1 minute. After 20 seconds of the incubation, the rate of the Ca accumulation suddenly decreases until saturation is attained which occurs at about 2 minutes after the start of the incubation. When saturated, the amount of the Ca accumulated under the conditions of the present measurement reached as much as about 60 mμmoles per mg of the microsomal protein. The conditions of the present measurement were those under which a maximal amount of Ca uptake was obtained as determined in preliminary experiments.

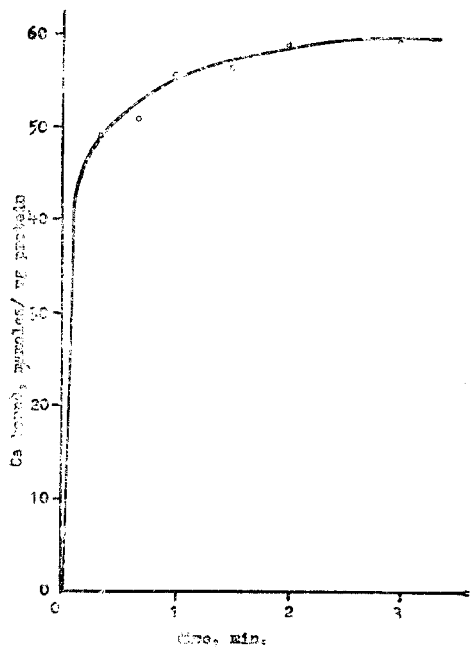

Fig. 1. The rate of Ca uptake of the fragmented sarcoplasmic reticulum of the rabbit skeletal muscle. The incubation medium consisted of the basic medium containing 2 mM $MgCl_2$ and 2 mM ATP. The concentration of the microsomal protein in the reaction mixture was 0.87 mg/ml.

2. The effects of K and Mg on the accumulation of Ca in the microsomes.

The Ca uptake by the fragmented sarcoplasmic reticulum was measured under various concentrations of K and Mg. The effect of ATP under these ionic concentrations was also studied, and the results are presented in Fig. 2. Microsomes were incubated in the basic medium in which K or Mg in the concentrations given in the legend of Fig. 2 were contained in the presence or in the absence of ATP (2 mM).

With no ATP present in the medium, the Ca accumulation by the microsomes decreases with increasing concentrations of K. In the presence of 50 mM KC1, which is the lowest concentration of K employed in the present experiment and which is the concentration originally contained in the basic medium, about 8 mμmoles of Ca were accumulated per mg of microsomal protein in a minute. This amount kept decreasing as the concentration of K increased above 50 mM. When ATP in the concentration of 2 mM was present in the medium,the amount of accumulated Ca was greater than in the absence of ATP and did not decrease with the increasing K concentration. Thus, the presence of ATP in the medium prevented the inhibiting effect of K on the microsomal Ca uptake.

The presence of Mg in the basic medium seems to inhibit the uptake of Ca by the microsomes when no ATP is present. This inhibiting effect of Mg is greater than that of K. As shown in Fig. 2, the amount of Ca in the basic medium which lacks Mg is about 7 mμmoles/mg protein in a minute. When Mg is present in the concentration of 1 mM, the amount of the Ca accumulation decreases and keeps decreasing until the concentration of Mg is increased up to 5 mM. Higher concentration of Mg than 5 mM seems to have no further effect. The presence of 5 mM Mg in the basic medium decreased the Ca accumulation to less than half the value obtained when no Mg was present.

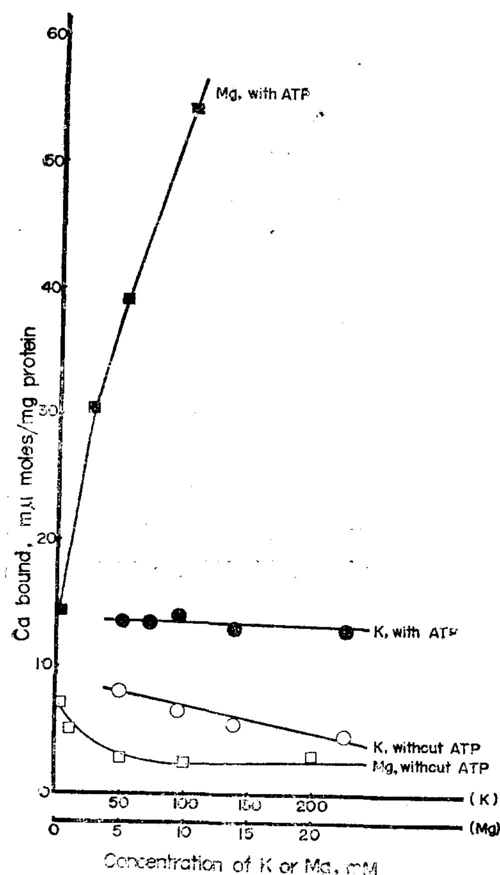

Fig 2. The effects of K and Mg, in the presence and in the absence of ATP, on the Ca uptake of the fragmented sarcoplasmic reticulum of the rabbit skeletal muscle. Microsomes were incubated in the basic medium containing K or Mg in the concentrations given in the Fig. The concentration of ATP when present was 2 mM.

In the presence of 2 mM ATP in the medium, on the other hand, Mg increases the Ca uptake of the microsomes greatly. The higher the concentration of Mg in the range between 1 to 10 mM, the greater is the Ca uptake. When no Mg is present, the presence of ATP causes an uptake of Ca of about 15 mμmoles/mg protein. When Mg is added to this system in the concentration of 2.5 mM, the Ca uptake is greatly enhanced and more than twice the amount of Ca is accumulated. When 10 mM Mg is present, the Ca accumulation is far greater; it reaches more than 54 mμmoles/mg protein which is more than 20 times that obtained when no ATP is present. The presence of ATP along with Mg is thus far more effective on the Ca accumulation than the coexistence of ATP and K.

3. The effects of K and Mg on the ATPase activity of the microsomes.

Since the Ca uptake by the microsomes against concentration gradient must depend on the hydrolysis of ATP (Fig. 2), the ATPase activity of the microsomes under various concentrations of K and Mg were measured in the presence of 2 mM ATP in order to link this activity with the Ca uptake under these conditions and the results are shown in Table 1. The concentrations of K in the medium affect little

the ATPase activity of the microsomes. The ATPase activity was about 80 mμmoles Pi/mg protein/min. regardless of the concentration of K in the medium. The ATPase activity in the complete absence of K was not measured since the basic medium originally contained 50 mM K.

When Mg is present, the ATPase activity increases greatly. As shown in Table 1, the presence of 2.5 mM Mg causes the ATPase activity about 180 mμmoles Pi/mg protein/min., a value which is nearly twice that found in the absence of Mg. The concentration of Mg in the medium, however, seems to have but a negligible effect on the ATPase activity as in the case of K. Thus, a concentration of 2.5 mM of Mg gives almost the same activity of ATPase with 5 or 10 mM.

Table 1. The effects of K and Mg on the ATPase activity of the fragmented sarcoplasmic reticulum of the rabbit skeletal muscle.

Ion		ATPase activity, mμmoles Pi/mg protein/min
	138 mM	81.7
K^+	94	78.7
	72	83.7
	50	85.1
	10	184.9
Mg^{++}	5	188.9
	2.5	180.6
	None	92.0

4. The effects of caffeine, procaine and quinine on the accumulation of Ca in the microsomes.

The Ca uptake by the microsomes in the presence of ATP was measured under various concentrations of caffeine, procaine and quinine to determine the effects of these drugs on the Ca accumulation of the microsomes and the results are summarized in Table 2. The incubation media consisted of basic medium containing 7.5 mM $MgCl_2$, 1 or 2 mM ATP and caffeine, procaine or quinine in the concentrations as specified in the Table.

The presence of caffeine in the concentrations of above 5 mM inhibits the Ca uptake significantly. Low concentrations of caffeine (lower than 5 mM), however, has little or no effect on the uptake.

A similar pattern of decreasing effect is also seen in the media containing procaine (procaine hydrochloride). Procaine in the 1 mM concentration decreases the Ca uptake and this decreasing effect is more significant as the procaine concentration increases. At lower concentration of ATP (1 mM), this decreasing effect is less obvious pre-

Table 2. The effects of caffeine, procaine and quinine on the Ca uptake of the fragmented sarcoplasmic reticulum of the rabbit skeletal muscle. The medium contained 7.5 mM $MgCl_2$ and 1 or 2 mM ATP

Drug		Ca accumulated, mμmoles/mg protein	
		ATP, 1 mM	ATP, 2 mM
Caffeine	10 mM		27.18
	5		17.13
	2.5	21.50	34.23
	1.0	26.15	40.78
	None	28.45	36.80
Procaine	10 mM		25.45
	5		29.65
	2.5	28.55	31.20
	1.0	15.85	31.30
	None	29.35	38.22
Quinine	0.25 mM		32.42
	0.125		35.45
	0.0625	22.90	35.60
	0.025	25.90	39.63
	None	25.85	36.03

sumably because of the smaller accumulation of Ca by the microsomes at this concentration of ATP.

Unlike the effects of caffeine and procaine, the presence of quinine (in the form of quinine sulfate) in the range between 25 to 250 μM has practically no effect on the Ca uptake of the microsomes. The concentrations of quinine employed in the present measurement were not the same with those of caffeine and procaine and were much lower than the latters simply because of its solubility in the basic medium. It was observed that ATP caused greater accumulations of Ca in the media containing these drugs. Higher ATP concentration enhanced the Ca uptake.

5. The effects of caffeine, procaine and quinine on the ATPase activity of the microsomes.

Table 3 shows the ATPase activity measured in the media containing various concentrations of caffeine, procaine or quinine. The media contained 7.5 mM $MgCl_2$ and 1 or 2 mM ATP to give a maximal activity of the enzyme.

Table 3. The effects of caffeine, procaine and quinine on the ATPase activity of the fragmented sarcoplasmic reticulum of the rabbit skeletal muscle. The media contained 7.5 mM $MgCl_2$ and 1 or 2 mM ATP

Drug		ATPase activity, mμmoles Pi/mg protein/min.	
		ATP, 1 mM	ATP, 2 mM
Caffeine	2.5 mM	225	231
	1.0	227	226
	None	227	218
Procaine	2.5 mM	205	185
	1.0	247	185
	None	225	186
Quinine	0.0625 mM	214	234
	0.025	208	242
	None	218	279

Unlike the effects of these drugs on the Ca uptake of the microsomes, neither one of the three drugs has any effect on the ATPase activity of the fragmented sarcoplasmic reticulum.

DISCUSSION

In the present experiment, the Ca uptake by the fragmented sarcoplasmic reticulum of the rabbit skeletal muscle was almost completed within one minute of the incubation. As shown in Fig. 1, nearly 80% of the saturation value of Ca uptake occurred within 20 seconds and the rate decreased greatly thereafter. At 1 minute of the incubation about 95% of the saturation value was accumulated in the microsomes indicating that the saturation occurs at about 1 minute.

There are numerous reports concerned with the rate and time course of Ca uptake by the microsomes. Carsten and Mommaerts (1964) reported that in the presence of 1 mM ATP and 1.25 μM Ca (per mg of microsomal protein) the uptake was almost complete in 8 minutes with 0.08 mg microsomal protein/ml incubation mixture. The Ca concentration in the incubation medium they employed is very much lower than that of the present experiment which used 0.2 mM. Even considering the low concentration of the microsomal protein in the incubation medium they used, their Ca concentration is far below that of the present study. The relatively long period of time required for the saturation of the Ca uptake they reported would then be consi-

dered to be due to this low concentration of Ca; the lower the concentration of Ca in the surrounding medium, the longer would be the time required for the microsomes to attain the saturation of Ca uptake since passive diffusion of Ca into the microsomes from the medium would play little contribution to the accumulation of Ca in the microsomes in such conditions. Although they reported that the saturation required more than 2 minutes regardless of the microsomal concentration, even in the high concentration ranging from 1 to 3 mg/ml, it is still longer than the time measured in the present study indicating that the saturation time depends on the concentration of Ca in the medium. Hasselbach and Makinose (1962) and Martonosi and Feretos(1964 a,b) also reported that the saturation of the uptake occurred within 2—15 minutes. Further, Ebashi and Yamanouchi (1964) reported that in the presence of 0.1 mM Ca the saturation took 2—3 minutes, and Ha (1967) obtained the same value with the same concentration of Ca. These results could also be accounted for by the same consideration as described above. On the other hand, Ohnishi and Ebashi (1964) reported that the saturation was completed instantaneously, a result which is in good concordance with the present one.

Concentrations of the microsomal protein also seem to affect the saturation time of Ca uptake. Takauji *et al.* (1965) measured the saturation time with various concentrations of the microsomal protein in the medium containing 0.2 mM Ca and reported that the saturation occurred at about 2, 5, and 15 minutes for 0.2 mg, 0.5 mg and 1.0 mg of protein/ml of reaction mixture, respectively. These results also support the present interpretation for the discrepancies regarding with the saturation time.

Various authors have studied the effects of K and Mg on ATP-supported Ca uptake by the skeletal muscle microsomes with contradictory results. Martonosi and Feretos (1964 a), with the use of 4 mM ATP, have found that increasing the ionic strength of the medium from 0 to 0.2 M with KCl caused a slight decrease of the Ca uptake. Under similar experimental conditions, Leopoldo (1969) has obtained the same results. Cohen and Selinger (1969) also found that the Ca binding decreases progressively in the presence of increasing concentrations of KCl. A leveling off of Ca binding on increasing the concentration of K can also be seen in the experiments of Carvalho(1966), who reported that Ca, Mg and K are bound to the same sites on the reticular membrane with the order of affinity of Ca>Mg>K. He concluded that in the presence of K or Mg, the apparent affinity of the microsomes for Ca decreases because of the competition between the two cations for the same binding sites. His experiments were, however, carried out in the absence of ATP and consequently his interpretation is restricted to the elucidation of the physicochemical interaction of the microsomal material with Ca and other cations. In the presence of ATP, Carvalho (1967) found that the affinity of surface-binding sites for Ca are greatly increased.

On the other hand, Rubin and Katz(1967) have found that K, in the concentrations up to 120 mM, enhanced Ca uptake. Ohtsuki (1969) reported that in the dog brain

microsomes the Ca uptake was dependent on the Mg concentration. He observed that very small amount of Ca uptake was observed without the addition of Mg ions and this amount was increased greatly by the increase of Mg ions from 0 to about 2 mM. When the Mg ion concentration exceeded 2 mM, a slight decrease in the Ca uptake was observed. However, at the concentrations of Mg higher than 5 mM, there was no appreciable change in the Ca uptake. Ogawa (1970) found that both the capacity and the rate of Ca uptake depended on the concentration of free Mg and Mg-ATP, and that the capacity and the rate reached the maximum at 1 mM and 3 mM Mg, respectively.

In the present experiment, the Ca uptake kept decreasing as the concentration of K in the medium increased from 50 mM to 225 mM in the absence of ATP (Fig. 2). In the presence of 2 mM ATP, however, the Ca uptake remained essentially the same regardless of the increased K concentrations. It is, therefore, considered that in the absence of ATP, K competes with Ca for the same binding sites on the microsomes, as Carvalho considered, but, in the presence of ATP, the membrane selectively binds Ca even in the presence of high concentration of K. This is more apparent when one observes the effects of Mg on the Ca uptake (Fig. 2). Here, Mg inhibits the Ca uptake by the microsomes if no ATP is available in the medium. On the other hand, when ATP is present in the concentration of 2 mM, the presence of Mg greatly enhances the Ca uptake. This is interpreted as because the Mg activates the ATPase of the microsomes and induces a great deal of Ca accumulation. This view is supported from the results of the measurements of the ATPase activity of the microsomes (Table 1), which reveal that more than twice activity was observed in the presence of Mg. From Table 1, it is also apparent that the ATPase activity of the microsomes is influenced little by the concentration of K, while it is greatly increased with the presence of Mg. Several authors, with the use of ATP as a substrate, have shown that the Ca transport is associated with the Ca-activated ATPase (Hasselbach and Makinose, 1961; Martonosi and Feretos, 1964 b; Ha, 1967; Leopoldo, 1969).

From these considerations, it is concluded that the membranes may have common binding sites for Ca, K and Mg, but the presence of ATP activates the membrane ATPase increasing the Ca binding.

In the present experiment, a part of the Mg added might have bound with ATP to form Mg-ATP, because the concentration of ATP in the form of disodium salt was as high as 2 mM. Consequently, the Ca uptake can be explained to increase in the presence of Mg of even 10 mM.

Bianchi (1963) reported that caffeine increases the outflux of Ca from sartorius muscle presumably by causing the release of Ca bound to the membrane of sarcoplasmic reticulum. Carvalho (1966) investigated whether caffeine interferes with the binding of Ca at the anionic sites of the fragmented sarcoplasmic reticulum and reported that this drug (10 mM) had no effect on the Ca bound by the microsomes under the conditions in which 0.7 mM Ca was used. His experiments were done on

the microsomes preloaded with Ca and measuring the amount of the Ca released on the treatment of the caffeine. He concluded that caffeine does not compete with Ca for the anionic binding sites of the sarcoplasmic reticulum. There is indication from studies on the effect of caffeine on the fraction of Ca uptake by the microsomes which is ATP, dependent that the effect of caffeine on muscle contraction might be mediated through that fraction of Ca which is actively accumulated by the sarcoplasmic reticulum (Herz and Weber, 1965).

The results reported here show that either caffeine and procaine, but not quinine depresses the binding of Ca by the microsomes induced by ATP (Table 2). The interference with binding of Ca caused by caffeine is consistent with the suggestion of Bianchi (1963) that the release of Ca bound to the microsomal membranes is caused by caffeine. It is, therefore, concluded that caffeine produced contracture through an inhibition of sarcotubular Ca transport. This view is also supported by the study of Fuchs (1969), who reported that caffeine (1—10 mM) inhibits the Ca uptake. Similar results are also found in the study of Ogawa (1970). He reported that the caffeine is considered to be the releaser of accumulated Ca rather than the inhibitor of Ca uptake. In the present study, however, it is apparent that the caffeine does inhibit the Ca uptake.

Procaine, which is also known to cause contracture of skeletal muscle of frog and rat (Bianchi and Bolton, 1967), may also release the Ca bound to the membranes. The effects of this substance (Table 2) can be interpreted in the same way as that of caffeine.

Quinine is reported to release from the membranes passively bound Ca; i.e., Ca bound nonspecifically in the absence of the added ATP (Carvalho, 1968 a,b). The Ca accumulated in the microsomes by an active mechanism, probably by the active transport mechanism, in the presence of ATP is not released by quinine(Carvalho, 1968 b). In the preseat experimeut, the effect of quinine on the Ca uptake was measured in the presence of 1 or 2 mM ATP, and no appreciable effect was observed. If the Ca taken up "actively" is not released as Carvalho (1968 b) concluded, the results of the present study on the effect of quinine on Ca uptake may be interpreted as due to the presence of ATP in the medium.

Unexpectedly, the ATPase activity of the microsomes was not affected by caffeine, procaine or quinine(Table 3). Since quinine is con sidered to cause the release of the fraction of Ca that is bound "passively" to the membrane, and since the ATPase may not be involved in this passive process, quinine seems not to be effective on the ATPase activity of the microsomes. In fact, quinine did not inhibit the Ca uptake of the microsomes measured in the presence of ATP (Table 2). The results obtained for caffeine and procaine are difficult to explain at present. They caused the decrease in the Ca uptake, not affecting the ATPase activity of the membranes. It seems that these potentiators inhibit the Ca transport through unknown mechanisms, not through the ATPase activity.

SUMMARY

The Ca uptake by the fragmented sarcoplasmic reticulum of the rabbit skeletal muscle was measured under various concentrations of K, Mg, caffeine, procaine and quinine. The ATPase activity of this reticular membrane was measured under the same conditions simultaneously.

The saturation of Ca uptake was almost completed within 1 minute. The Ca uptake was inhibited by high concentrations of K (above 50 mM) and Mg (above 1 mM) in the absence of ATP. When ATP is present, however, the Ca uptake did not reflect the concentration of K, while it increased greatly as the concentration of Mg was increased. Caffeine and procaine caused the inhibition of Ca uptake in the presence of ATP, but quinine did not.

The ATPase activity of the membrane was little affected by the concentration of K, while it was enhanced in the presence of Mg. Caffeine, procaine and quinine did not influence the ATPase activity.

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