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EFFECT OF POTENTIATORS OF MUSCULAR CONTRACTION ON CONTRACTILE AND ENZYMATIC ACTIVITIES OF SARCOLEMMA

ARSELIO P. CARVALHO, VITOR M. C. MADEIRA AND M. C. ANTUNES-MADEIRA

Institute for Muscle Disease, New York, N.Y. 10021 (U.S.A.) and Department of Zoology, University of Coimbra, Coimbra (Portugal).

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SUMMARY

Sarcolemma tubes isolated from rabbit skeletal muscle exhibit a predominantly radial contraction which can be induced by ATP, Zn^{2+}, and Cd^{2+} and to a smaller extent by ADP, SCN^-, Ca^{2+} and Mg^{2+}. The contraction is an intrinsic property of the sarcolemma, since it cannot be attributed to osmotic effects or to the activity of adhering myofibrils. Parallel studies on the surface ATPase activity of isolated semitendinosus muscles of the frog show that this enzymatic activity is inhibited reversibly by Zn^{2+} or Cd^{2+}. The results are related to the twitch potentiating effects of some of the substances tested.

INTRODUCTION

The force of contraction developed during the twitch of skeletal muscle cells is increased by the presence in the medium of substances known as potentiators[1,2]. These substances vary widely in chemical nature (*e.g.*, Zn^{2+}, SCN^-, caffeine) and no unified theory of their mode of action exists presently. At least some of these substances appear to act at the surface of the muscle cell since their effect either occurs too rapidly to permit penetration (NO_3^-, I^-, SCN^-) or it is readily reversible (*e.g.* Zn^{2+}, Cd^{2+}) by washing with Ringer's solution containing EDTA which does not penetrate the muscle cell[3,4].

Clearly it is of interest to study the interaction of these substances with the sarcolemma. This type of study has become possible only in recent years with the development of techniques for the isolation of sarcolemma[5–9]. In the present study we have used two approaches to the problem: We studied the interaction of potentiators and other substances of physiological interest (*i.e.* ATP, Ca^{2+}, Mg^{2+}, K^+, *etc.*) with fragments of isolated sarcolemma tubes, and tested the effect of some of these agents on the activity of the ATPase which recently was shown to be bound very tightly at the surface of skeletal muscle cells[10–12].

Sarcolemma tubes isolated from rabbit skeletal muscle are shown to contract radially and this contraction can be induced by either ATP, ADP, or by the potentiating agents Zn^{2+}, Cd^{2+}, SCN^-, and only to a very small extent by Ca^{2+} or Mg^{2+}. In a parallel study we found that the surface ATPase activity of frog skeletal muscle cells is stimulated by Mg^{2+} and inhibited by the cationic potentiators Zn^{2+} or Cd^{2+}.

We relate these surface phenomena of the skeletal muscle cell to its physiological functions.

METHODS AND MATERIALS

Preparation of biological material

The isolated sarcolemma employed in this study were obtained from the back and leg muscles of the rabbit by a slight modification of the method of ROSENTHAL *et al.*[9]. The method which includes physicochemical manipulations, permits the emptying of the muscle cells such that the myofibrils, nuclei, mitochondria, and other cellular components are extruded and leave a relatively transparent and empty tube which presumably consists of the sarcolemma. This tube can be seen with the aid of the electron and phase contrast microscopes[9,13]. The extrusion of the intracellular components of the muscle cells is induced by a sequence of treatments of the muscle homogenate prepared originally in 50 mM $CaCl_2$. The homogenate is washed three times with KCl buffer (50 mM KCl + 30 mM $KHCO_3$ + 2.5 mM histidine at pH 7.6), and is then incubated at 37° for 30 min. This incubation apparently destroys the cytoskeletal of the cells so that the intracellular components can now be removed by washing with $2.5 \cdot 10^{-7}$ M NaOH. The sarcolemma are then separated from the other cellular components by differential centrifugation and kept suspended in distilled water or 5 mM imidazole at a pH value of 7.0. (For details see ROSENTHAL *et al.*[9] and MADEIRA AND CARVALHO[13].) When resuspended in distilled water the sarcolemma maintain their visible structure for longer periods (2–3 days) than when suspended in the imidazole buffer. Therefore, we routinely kept the isolated sarcolemma in water at 4° and added the buffer just before the studies performed with them were carried out. The sarcolemma are easily seen with the aid of a phase contrast microscope at a magnification of 200 × if a drop of sarcolemma suspension is added to a glass slide and a cover slip is placed over the drop. These preparations were viewed with a Nikon microscope and photographed with an Asahi Pentax SV camera using Kodak Panatomic *X* film. For the enzyme studies, isolated semitendinosus muscles of the frog (*Rana ridibunda*) were used. The muscles were dissected and kept for 10–15 h at 3–5° before they were used in the experiments. The muscles were kept in 20 ml of the following solution: 2.5 mM KCl, 117 mM NaCl, 10 mM imidazole (pH 7.0) and 1–2 mM. $CaCl_2$. This solution is henceforth referred to as Ringer–imidazole solution.

Methods of study of the isolated sarcolemma

The methods employed in this study were essentially those described previously in a study of some morphological effects of ATP, ADP and AMP on isolated sarcolemma[13]. Since the method imposes certain limitations which should be kept in mind in interpreting the results reported, we describe it briefly here.

The experiments were carried out at 20° on drops of buffered suspensions of sarcolemma at pH 7.0 placed under a cover slip on a glass slide. The test solutions were then added by placing them in the periphery of the cover slip so that they diffuse under it and mix with the sarcolemmal suspension. As an index of this diffusion we used the movements of visible particles that are usually present in the suspension under the cover slip. The diffusion is relatively rapid since it usually takes less than 10 sec for a response to be elicited on the sarcolemma.

This method does not permit rigorous control of the concentration of test substances in the vicinity of the sarcolemma, but it permits us to carry out comparative studies of the effects of the various substances tested on the contractility of the sarcolemma since in each case we started the experiment with the same concentration of the substances to be compared. The differences in diffusion rates of the various substances tested is of little importance in these studies since we measured the effects after a relatively long period (1–5 min) after the addition of the test substances.

Methods of equilibrating and washing the isolated semitendinosus muscles for enzymatic studies in situ

In view of the uncertainty regarding the purity of the isolated sarcolemma, we felt that enzymatic studies with these preparations were not justified at this time. We cannot exclude the possibility that a very small amount of myofibrils or actomyosin remains adhered to the sarcolemma tubes, and, therefore, the significance of the results of enzymatic studies of these tubes would remain highly questionable, particularly since we were interested in the ATPase activity of the sarcolemma and as is well known the contractile proteins catalyse the hydrolysis of ATP under various

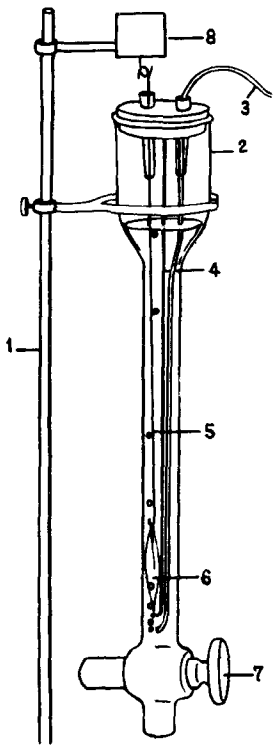


Fig. 1. Chamber of approx. 2 ml capacity used to equilibrate the muscle during the studies on the surface ATPase activity of the semitendinosus muscle of the frog. The numbers refer to the various parts of the chamber and accessories. 1, holding chamber. 2, Pyrex chamber; volume of narrow part of chamber is 1.5 ml. 3, tube for bubbling air. 4, stainless steel rod with hook at the lower end. This rod is secured at the top of the chamber. 5, stainless steel wire with hooks at both ends to connect the muscle to transducer. 6, semitendinosus muscle. 7, stopcock. 8, transducer.

conditions[14]. After this work had been completed a report appeared which presents evidence that by a technique only slightly different from that employed by us it is possible to prepare sarcolemma tubes with a ($Na^+ + K^+$)-ATPase which is not characteristic of other cell fractions isolated from muscle[31]. Enzymatic activity of the sarcolemma could be studied *in situ* as had been shown by other workers[10,11]. Some of the results obtained in this study have been presented preliminarily[12].

The chamber used to carry out these studies is illustrated in Fig. 1. After the muscle is inserted in the chamber the wash and test solutions are introduced through a hole in the stopper at the top of the chamber. A tube of polyethylene is inserted through a second hole in the stopper and air or oxygen is bubbled at a constant rate so that the solution in the chamber is aerated and also mixed constantly.

All experiments were carried out for varied periods at 20° with 1 ml of the desired solution. At the end of each experiment, if the muscle was to be used again, it was washed with Ringer–imidazole solution by filling the chamber with this solution and immediately discarding the wash. To the chamber were then added 2–3 ml of the Ringer–imidazole solution which was allowed to stay in contact with the muscle for about 5 min. This short equilibration was repeated once more and was usually found to reverse most effects on the surface enzymes induced by the substances tested in this study. In those studies in which the activating effect of Mg^{2+} was studied the Ca^{2+} was left out of the Ringer–imidazole solution. We observed in preliminary experiments that stretching the muscle to various lengths did not have any measurable effect on the surface ATPase activity. Therefore, all studies reported here were carried out at rest length.

Phosphate analyses

The amount of inorganic phosphate (P_i) produced from ATP as it came in contact with the muscle was determined by the method described by TAUSSKY AND SHORR[15].

RESULTS

Contractility of isolated sarcolemma

The isolated sarcolemma appear as relatively empty fragments of tubes of 50–100 μm in diameter when observed by phase contrast microscopy, as shown in Fig. 2A. In general appearance, these tubes are similar to those reported by other authors[5–9]. The fragmented tubes usually appear open at both ends, but occasionally one of the ends tapers off in a cone shaped configuration which probably represents one end of the original muscle cell.

We established previously[13] that isolated sarcolemma contract when a solution of 2–10 mM ATP is applied to the preparation as described in METHODS AND MATERIALS. The contraction induced by ATP does not require Ca^{2+} nor is it inhibited by high ionic strength (1 mM KCl)[13] and, therefore, it depends on a mechanism which is different from that responsible for the contraction of actomyosin systems[14,16,17]. Figs. 2A and 2B show that 2 mM ATP induces radial contraction of the sarcolemma in the presence of 10 mM EGTA which chelates traces of Ca^{2+} that may be present in the medium. These conditions, and those in which 1 M KCl is present, inhibit all myofibrillar and actomyosin activity[14,16,17], whereas the radial contraction of the

sarcolemma induced by ATP actually is accentuated by EGTA even though this substance alone is without effect[13]. ROSENTHAL *et al.*[9] apparently were the first investigators to observe that isolated sarcolemma contract when ATP is added, but no particular significance was attributed to this phenomenon.

Since various cellular membrane fragments form closed vesicles (*e.g.* vesicles of isolated sarcoplasmic reticulum) which may behave as osmometers, we studied whether the sarcolemma tubes behave in a similar fashion. These studies were carried out by varying the osmotic pressure of the medium around the isolated sarcolemma and observing the alterations in their volume with the aid of a phase contrast microscope which permitted the sarcolemma to be photographed under various conditions. The solutions tested were KCl (0.01 M, 0.1 M and 1 M) and sucrose (0.1 M, 0.3 M, and 1.0 M), and no osmotic effects were observed either in hypotonic or in hypertonic solutions (Table I). Therefore, we concluded that K^+, Cl^- and sucrose enter the sarcolemma tubes very rapidly, probably through the cut ends which apparently do not reseal, and that the contraction of the isolated sarcolemma referred to above represents an inherent property of the isolated structures.

Although isolated sarcolemma have been reported to have ATPase activity[18–20,31], the contractility is not necessarily mediated by the hydrolysis of ATP. It is conceivable that the mere interaction of the nucleotide with the sarcolemma causes the alterations observed.

TABLE I

SUMMARY OF THE QUALITATIVE EFFECTS OF VARIOUS SUBSTANCES ON THE CONTRACTILE ACTIVITY OF SARCOLEMMA ISOLATED FROM RABBIT SKELETAL MUSCLE

Substance tested	*Concentration (mM)*	*Effect on isolated sarcolemma*
Adenine nucleotides		
ATP	10	Strong radial contraction
ATP	2–5	Contraction not always observed
ATP + EGTA	2–5 + 10	Strong radial contraction always observed
ADP	10	Radial contraction smaller than that observed with 10 mM ATP
AMP	10	No visible effect observed
Potentiators of muscular contraction		
Zn^{2+}, Cd^{2+}	5	Strong radial contraction
SCN^-	100	Small radial contraction
I^-, Br^-, NO_3^-	100	No visible effect observed
Caffeine	10	No visible effect observed
Some ions of physiological importance		
Ca^{2+}, Mg^{2+}	10	Small radial contraction
Ca–EGTA	5	No visible effect observed
Ca^{2+} or Mg^{2+} + ATP	10 + 10	Strong radial contraction
Ca–EGTA + ATP	5 + 10	Strong radial contraction
KCl or NaCl	0.01, 0.1 and 1 M	No visible effect observed
KCl + ATP	1 M + 10 mM	Strong radial contraction
Other substances tested		
Sucrose	0.1, 0.3 and 1 M	No visible effect observed
Glycerol	50%, 400 mM	No visible effect observed

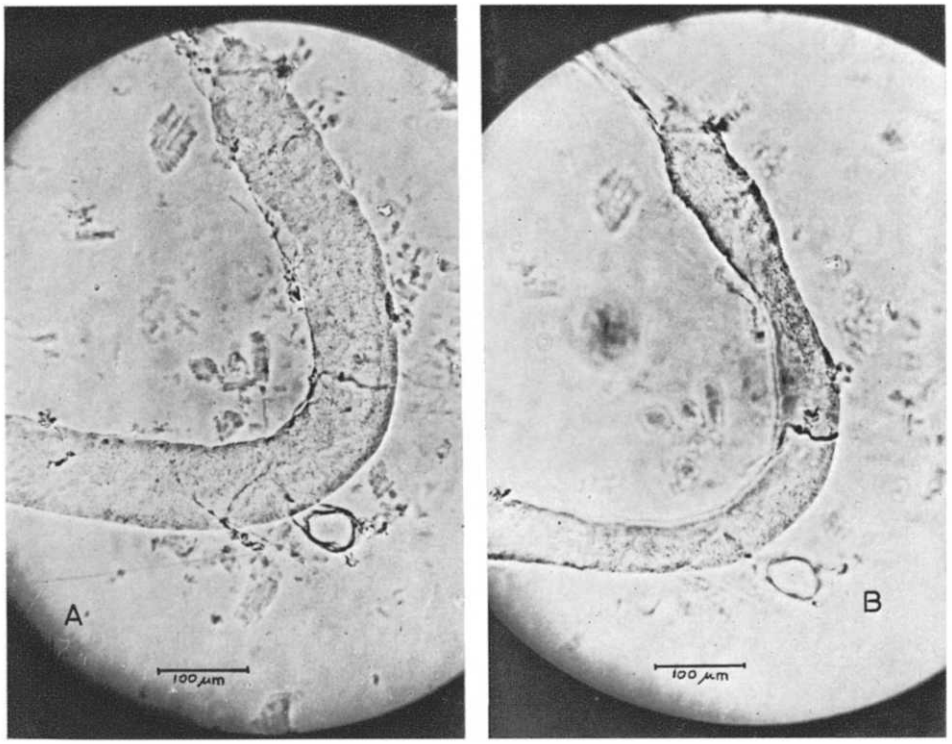


Fig. 2. Phase contrast micrographs of tubes of sarcolemma isolated from rabbit skeletal muscle. A. Normal appearance of the sarcolemma. B. Appearance of sarcolemma after radial contraction induced by 2 mM ATP in the presence of 10 mM EGTA.

Effects of potentiators of muscular contraction on isolated sarcolemma

Various substances increase the tension developed by skeletal muscle during the twitch. These substances, known as potentiators, include Zn^{2+}, Cd^{2+}, UO_2^{2+}, SCN^-, I^-, among others[1,2]. These potentiators probably act on or near the surface of the muscle cell since they either alter the pattern of the action potential (Zn^{2+} and Cd^{2+}) and their effect can be rapidly reversed if the muscle is placed in a normal Ringer's solution[1-3], or then their effect occurs too rapidly to permit penetration (*e.g.* I^-, SCN^-)[4]. Therefore, we studied the effect of the potentiators on the contractility of the isolated sarcolemma.

In the present study we observed that at concentrations of 5.0 mM Zn^{2+} or Cd^{2+} (Fig. 3), and to a much smaller extent Ca^{2+} or Mg^{2+} (Fig. 4), cause radial contraction of the sarcolemma. Although we do not know the exact values for the concentrations of the ions in the vicinity of the membranes, they probably were at least one order of magnitude lower than those of the concentrations added. After the addition of Ca^{2+} or Mg^{2+}, a fuller contraction could be induced by ATP as reported previously[13]. If in addition to Ca^{2+}, an equimolar concentration of EGTA was also added simultaneously, the small contractile effect of Ca^{2+} was not observed (Fig. 5B), but if the sarcolemma tubes were allowed to contract first, EGTA could not reverse the effect of any of the cations tested. Furthermore, 2 mM ATP induces contraction in a medium of equimolar Ca–EGTA (Fig. 5C), and the effect of ATP is usually more pronounced

in the presence than in the absence of EGTA. The contractions observed in all cases are predominantly radial in that the diameter of the sarcolemma tube becomes smaller, but a slight decrease in length of the tube is also observed in some cases.

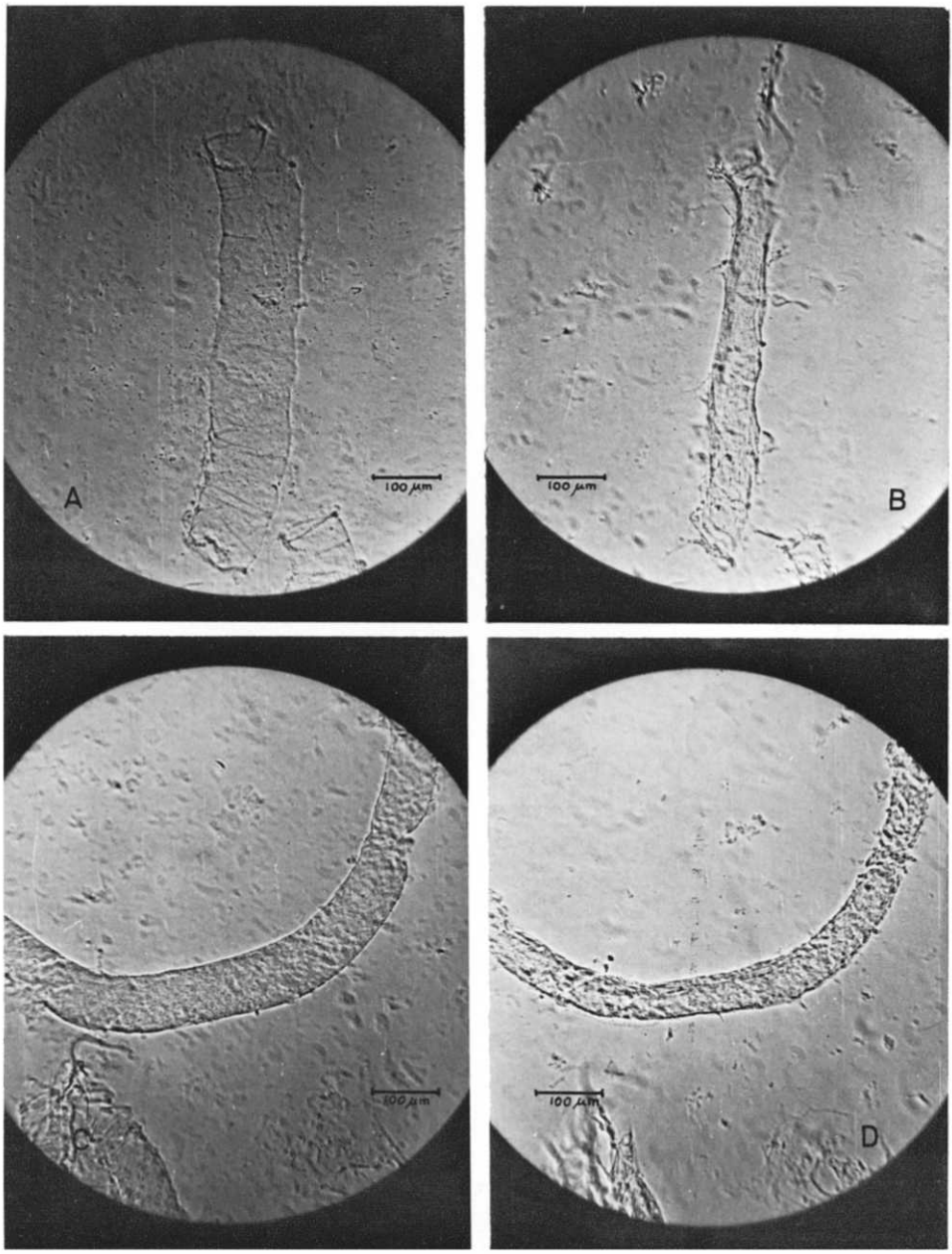


Fig. 3. Phase contrast micrographs of tubes of sarcolemma, isolated from rabbit skeletal muscle, before and after treatment with 5 mM Zn^{2+} or Cd^{2+}. A. Normal appearance of sarcolemma. B. Appearance of sarcolemma in A after treatment with Zn^{2+}. C. Normal appearance of another sarcolemma tube. D. Sarcolemma tube in C after treatment with Cd^{2+}.

The anionic potentiators of twitch tension, SCN^- I^-, Br^-, and NO_3^-, were also tested for their effect on the contractility of isolated tubes of sarcolemma. The concentrations of the potentiating substances added were 100 mM in the potassium form. Only SCN^- appears to cause a slight contraction of the sarcolemma (Table I). The implication of these observations will best be understood when we succeed in defining the basic contractile mechanism of the sarcolemmal structure.

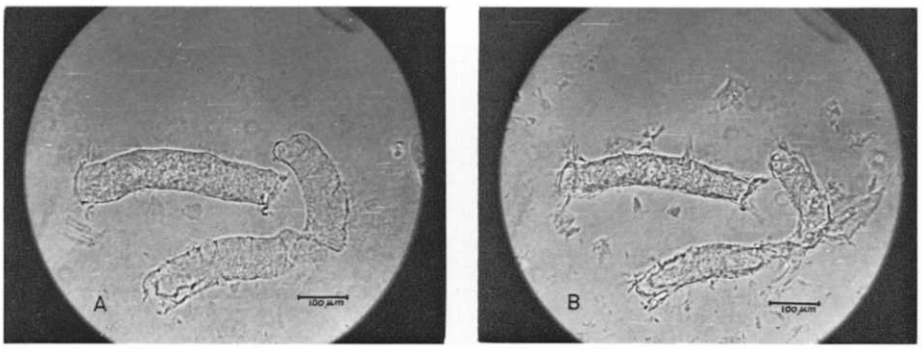


Fig. 4. Phase contrast micrographs of tubes of sarcolemma, isolated from rabbit skeletal muscle, before and after treatment with Mg^{2+}. A. Normal appearance of sarcolemma. B. Appearance of sarcolemma in A after treatment with 5 mM Mg^{2+}.

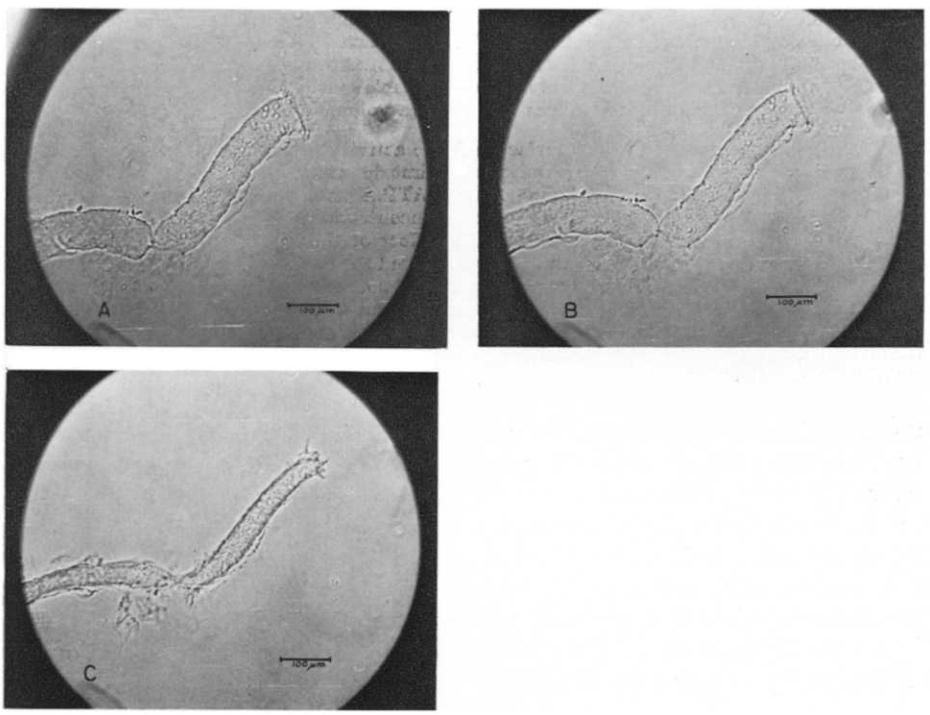
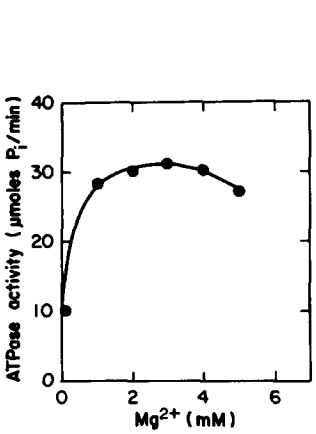


Fig. 5. Phase contrast micrographs of tubes of sarcolemma isolated from rabbit skeletal muscle. A. Normal appearance of sarcolemma. B. Appearance of sarcolemma after treatment with 5 mM Ca–EGTA. This solution has no visible effect. C. Appearance of sarcolemma after 2 mM ATP was added to preparation in B. Note the intense radial contraction.

Caffeine, a substance that also potentiates the twitch tension of skeletal muscle[1,2], but which presumably acts on the sarcoplasmic reticulum to release calcium[21–24], has no visible effect on the isolated sarcolemma at a concentration of 10 mM. Also, glycerol, which destroys the membrane structures of the muscle cell, does not produce any affect on the sarcolemma that can be discerned with the aid of the phase contrast



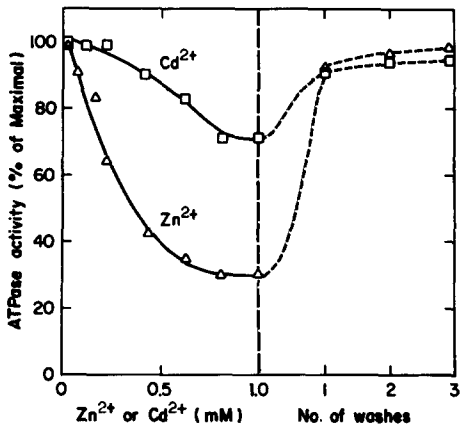


Fig. 6. Effect of Mg^{2+} ($MgCl_2$) on the surface ATPase activity of the semitendinosus muscle of the frog. The reaction was carried out for 15 min at 20° in Ringer–imidazole solution in which Ca^{2+} was omitted. The muscles were exposed to the lowest Mg^{2+} concentration first and then to each of the solutions containing the higher concentrations of the cation in an increasing sequential order. However, the solution at zero added Mg^{2+}, and which contained also 1.0 mM EDTA to complex trace amounts of Mg^{2+} was the solution tested last. After the exposure to each solution, the muscles were washed with the modified Ringer–imidazole solution (no Ca^{2+} present) as described in the text. The ATP concentration was 2.0 mM in a volume of 1.0 ml.

Fig. 7. Effect of Zn^{2+} and Cd^{2+} on the surface ATPase activity of semitendinosus muscle of the frog. The surface ATPase activity of the muscle was first determined at 20° in Ringer–imidazole solution (2.0 mM of Ca^{2+} present) containing 2.0 mM ATP. This activity was taken as 100% and the ATPase activity in the presence of the various concentrations of Zn^{2+} or Cd^{2+} added to the Ringer–imidazole solution was then measured. The effect of the lowest concentrations of each of the divalent cations was tested first, and then was tested the effect of the higher concentrations in increasing sequential order. After the test in the highest concentration of Zn^{2+} or Cd^{2+} (1.0 mM), the muscle was washed with Ringer–imidazole solution and the surface ATPase activity of the muscle was measured in this solution to see whether the effects of Zn^{2+} or Cd^{2+} could be reversed. Muscles could usually be used for several cycles without showing marked irreversibility of the effects of Zn^{2+} or Cd^{2+}.

microscope. The solutions of glycerol employed contained 50% glycerol, and were allowed to act on the membranes for period of 30–60 min. The lack of observable effect with glycerol is probably due to the fact that we cannot discern the various layers of the sarcolemma by phase contrast microscope, and, therefore, we are unable to detect any probable damage to the plasma membrane, which is the innermost layer of the sarcolemma[6,25], and is the likely target of glycerol. Furthermore, the period of 60 min may not be sufficient time for destruction of the membrane.

A summary of the results of the effects of the various substance tested on the isolated sarcolemma is presented in Table I.

Effects of Zn^{2+} and Cd^{2+} on the surface ATPase of frog skeletal muscle

There exists on the surface of frog skeletal muscles a bound ATPase which is not lost into the medium during equilibration of the muscles (semitendinosus) in

Ringer's solution[10–12]. This enzymatic activity is maximally stimulated by Ca^{2+} or Mg^{2+} at a concentration of 1–3 mM of either cation[12]. The evidence that the ATPase is on the surface of the muscle is reasonable only if we take as valid the reasonable assumption that ATP does not penetrate the cell membrane. The possibility that the enzyme is intracellular, but leaks out of the cell during equilibration, was eliminated in our studies, since the medium where a muscle has been equilibrated for various periods up to 1 h does not catalyze the hydrolysis of ATP after the muscle is removed[12]. Furthermore, no inorganic phosphorus appears in the medium during equilibration of the muscle unless ATP is present in the external medium.

Fig. 6 depicts the activation of the surface ATPase activity by Mg^{2+} in the presence of 2.0 mM ATP. Ca^{2+} acts similarly to Mg^{2+}, and at 2.0 mM of either cation in the Ringer's solution about the same activity of the ATPase was observed. The ATPase studied here is not sensitive to up to 10^{-5} M ouabain. These results agree with those reported by other investigators[10,11], but they are not conclusive in deciding whether a $(Na^+ + K^+)$-ATPase is present, since the bulk of the ATPase activity may in fact be ouabain insensitive, whereas only a small portion of the activity may be due to $Na^+ + K^+$ activation. Therefore, it is possible that the methods employed are not sufficiently sensitive to detect the $(Na^+ + K^+)$-ATPase.

Since Ca^{2+} is the normal divalent cation of Ringer's solution, we carried out the subsequent studies of the effects of Zn^{2+} and Cd^{2+} on the surface ATPase activity in the presence of 2.0 mM Ca^{2+}. This concentration of Ca^{2+} is slightly higher than that present in the bathing medium utilized in the studies on the effects of Zn^{2+} and Cd^{2+} on the contractile activity of the intact muscles[3,32]. We found that Zn^{2+} or Cd^{2+} in concentrations of about 0.05–1.0 mM inhibit the surface ATPase activity of frog skeletal muscle (Fig. 7). Invariably, the effect of Zn^{2+} is more pronounced than that of Cd^{2+}, but in both cases the effects are nearly completely reversible if the muscles are washed with Ringer–imidazole solution (Fig. 7).

All experiments were carried out with a concentration of ATP of 2 mM and only 15–20 % of this ATP was permitted to hydrolyze during the experiment. Considering the high activity of the surface adenylate kinase of the muscles used in these studies[10,11] most of the ADP resulting from the hydrolysis of ATP is converted to ATP. Therefore, the rate of appearance of P_i reflects closely the activity of the bound surface ATPase of the muscles.

The wet weight of the semitendinosus muscles employed in these studies varied between 29 and 55 mg. When expressed per unit area of cell surface the value for the ATPase of seven muscles varying in wet weight from 27 to 55 mg is 2.7 ± 0.6 nmoles P_i/cm^2 per min. The cell surface of the muscles was calculated from the relationship between the surface area of the cells and the wet weight of the muscle which was established by BIANCHI AND SHANES[26] for the sartorius muscle of the frog as being 300 cm^2/g wet weight of muscle.

DISCUSSION

The results reported here demonstrate that the isolated fragmented sarcolemma tubes display contractile activity which may be affected by the composition of the medium. Thus, ATP, Zn^{2+}, Cd^{2+} and, to a lesser extent, Ca^{2+}, Mg^{2+} and SCN^- induce a predominantly radial contraction of the sarcolemma tubes (Figs. 2–5). Such contrac-

tion, if it took place in the intact muscle cell, would be expected to cause elongation of the cell in order to maintain constant volume. In the present study elongation of the isolated sarcolemma is not observed probably because the fragmented pieces of the sarcolemma utilized were opened at both ends, and, therefore, the radial contraction would cause release of the fluid in the tubes through the cut ends. This could in fact be observed in the case of some fragments of sarcolemma which were not completely empty of solid material which could be seen coming out at either end of the tube during contraction.

The general appearance of the sarcolemma as seen with the aid of the electron microscope has been described[6,26]. The sarcolemma consist of three major layers, in addition to a superficial mesh of randomly arranged thin filaments; an outside collagenous layer, a middle amorphous layer (basement membrane) 300–500 Å thick, and the innermost layer which is interpreted as being the plasma membrane[26]. Since the contraction is essentially radial, we expect that there might be a defined orientation of the contractile components to permit this type of contraction. None of the layers of the sarcolemma has been described as having circular fibers, as would be necessary for radial contraction. Stepwise degradation of the sarcolemma by collagenase and a chloroform–ethanol mixture has been carried out successfully[6], and this approach may prove useful in future experiments to identify the layers that exhibit contractile activity.

The substances which induce contraction of sarcolemma are varied in chemical nature (Table I), and, therefore, we have no conceivable hypothesis as to the reactions involved. From the results of the cations tested, it appears that the stronger the affinity of the cation for biological membrane materials[27], the more pronounced is the effect of the cation. When ATP is the agent inducing contraction, a lower concentration of ATP is needed for maximal effect in the presence than in the absence of EGTA[13]. This suggests that EGTA may act by complexing an inhibitor of contraction normally present in the medium. Preliminary studies indicate that Ca^{2+} is not the inhibitor substance. In the absence of EGTA, some of the ATP may be tied up by complexing the inhibitor since it probably is a metal cation since other complexing agents (*e.g.* EDTA) have effects similar to those of EGTA. It should be noted that ATP is also a metal cation complexing agent and that its complexing properties have in fact been evoked to explain its role in membrane phenomena[28].

The inhibitory effects of Zn^{2+} and Cd^{2+} on the surface ATPase of the muscle cells may be of significance in relating the potentiating effects of these ions to the molecular mechanisms responsible for the potentiation, but no definite mechanism is proposed here. The active transport of Na^+ and K^+ is under the control of the well known ($Na^+ + K^+$)-ATPase, and recently it was suggested that the passive permeability of the muscle cell membrane of the crayfish may also be under the control of a Mg^{2+}-stimulated ATPase[29]. Thus, the potentiators could conceivably affect both the active transport and passive diffusion of Na^+ and K^+ across the muscle cell membrane. It may be significant in this respect that Zn^{2+} and Cd^{2+} prolong the repolarization phase of the action potential in the frog muscle[1,2].

Previous studies from our laboratory on other types of membranes isolated from skeletal muscle, *i.e.* sarcoplasmic reticulum[27], and current studies with isolated sarcolemma, show that Zn^{2+} and Cd^{2+} have an affinity for the membranous materials of muscle much higher than do other cations of physiological importance *e.g.* Ca^{2+},

Mg^{2+}, Na^{+} and K^{+}, and compete with these cations very effectively for the binding sites of the membranes. Thus, in addition to the effect on the ATPase, the binding of Zn^{2+} and Cd^{2+} to the sarcolemma in exchange for the other cations, may alter the permeability properties of the plasma membrane and lead to alterations in its electrical properties. This would in turn account for the potentiating action of Zn^{2+} and Cd^{2+}.

The physiological significance of the contractile property of the sarcolemma tubes is not clear. If the radial contraction observed in the isolated fragmented tubes is a normal function of the intact structure in the muscle cell under conditions of activation, the reduction in diameter of the cell caused by such contraction is expected to cause an elongation of the cell to maintain constant volume. This elongation could appear as the latency relaxation, assuming that the sarcolemma bears some of the resting tension as has been suggested by work from other laboratories[30].

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