Effects of Prostaglandins and Oxytocin on Calcium Release from a Uterine Microsomal Fraction

HYPOTHESIS FOR IONOPHORETIC ACTION OF PROSTAGLANDINS

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A microsomal fraction resembling striated muscle sarcoplasmic reticulum was isolated from uterine smooth muscle. ATP induces calcium accumulation in this fraction. Increased temperature enhances calcium accumulation and calcium-activated ATPase. In the absence of ATP, approximately 25% of the intracellular calcium exchanges with the 45Ca in the incubation medium. In the presence of ATP, exchange of intrinsic calcium with 45Ca increases by an amount which equals the ATP-dependent calcium binding. In preparations partially preloaded with calcium, a steady state of bound calcium is reached when the ATP is exhausted. Calcium is released under these conditions by prostaglandins E1 and F2α, but not by PGF2α. The antibiotic ionophores X537A and A23187, as well as oxytocin, also release calcium previously accumulated under ATP stimulation. None of these agents, with the exception of oxytocin, release intrinsic calcium. Thus, the effect of prostaglandins resembles that of the ionophores, suggesting an ionophoretic action of these prostaglandins. The release of calcium conforms with the in vivo smooth muscle contracting action of these agents.

The transport of calcium through the membranes of the sarcoplasmic reticulum is a well recognized step in the contraction and relaxation cycle of cardiac and skeletal muscle (1). Minute amounts of calcium are required for actomyosin activation in muscle contraction. This calcium is provided both by influx of extracellular calcium and by release from intracellular stores mostly in the sarcoplasmic reticulum. Relaxation is concurrent with a lowering of the intracellular free calcium concentration, the calcium being sequestered by the sarcoplasmic reticulum. Evidence for the role of intracellular calcium in contraction and relaxation of smooth muscle is based largely on the work of Van Breemen et al. (2); this involves the use of lanthanum to block the passage of calcium across the cell membrane. The presence of relatively large amounts of sarcoplasmic reticulum in uterine smooth muscle was established in electron microscope studies. It was shown that the volume of sarcoplasmic reticulum correlates with the dependence of the smooth muscle on intracellular calcium (3). Because the amounts of calcium involved appear to be much smaller than in other muscles, it is only recently that we have been able to demonstrate calcium accumulation on the subcellular level, particularly in uterine muscle (4).

It is thought that in muscle sarcoplasmic reticulum the measured calcium accumulation represents a steady state between active calcium accumulation and passive calcium efflux. Moreover, calcium binding can be reversibly displaced upon the addition of ATP (5, 6). We have been able to demonstrate energy-linked calcium accumulation in uterine microsomal preparations (4) as well as to show a dose-related inhibition of this calcium accumulation by uterine contractile agents such as certain prostaglandins and oxytocin (7). This phenomenon could be attributed to either an inhibition of ATP-dependent calcium binding or a simultaneous increase in hormone-induced calcium release.

In the present study we have attempted to separate these processes. Because of quantitatively improved calcium accumulation, it has been possible to show, in the present communication, the enhancement of calcium release by contractile agents in the microsomal model. In addition, we have considered the possibility that the prostaglandins act like ionophores.

MATERIALS AND METHODS

Method of Preparation—In brief, the procedure, modified from a previous study (8) is as follows: uteri, obtained at the slaughter house from close-to-term pregnant cows, were immediately dissected and the myometrium carefully stripped free of endometrium. The muscle strips were rinsed, immersed in ice-cold buffer (0.3 M sucrose, 5 mM dithiothreitol, 0.02 M Tris, pH 7.2), and transported in an ice bath to the laboratory. The muscle tissue was diced with scissors, minced in a meat grinder, and homogenized first in a Waring Blender for 15 s, then in a Polytron for 5 s, followed by a Waring Blender for 5 s. All operations were carried out in the cold room at 0-4°C. Differential centrifugation was at 2,500 x g for 20 min, 15,000 x g for 20 min, and 40,000 x g for 90 min. The final pellets were suspended in 0.08 M sodium oxalate and placed on a sucrose density gradient consisting of layers of 35, 45, and 55% sucrose. After 3 h of centrifugation in a Spincos 25.1 swinging bucket rotor (average force 64,000 x g), the main protein layer was isolated from the 35% sucrose layer (density of 1.136). Protein concentration was determined by the method of Lowry et al. (9). The protein was stored at 4°C and used the following day. The yield obtained was 122 ± 5.6 mg/kg of tissue, averaged for 34 experiments. Previous characterization (8) of this fraction showed no mitochondrial or myofibrillar contamination detectable by biochemical and morphological analyses. The latter showed predominantly vesicular structures, resembling frag-
ment of sarcoplasmic reticulum of cardiac muscle.

**Calcium Binding Centrifugation Method**—ATP-dependent calcium binding was assayed in incubation mixtures containing an excess of ATP. Reactions were started by addition of protein and terminated after specified times of incubation by plunging the tubes into an ice bath. In the centrifugation method (10) as modified by us (4), the tubes were centrifuged at 165,000 × g, for 30 min, in a Spincel T-60 rotor. The pellets were washed and homogenized in 20 mM imidazole buffer, and the centrifugation was repeated, all at 4°C. The pellets were then rehomogenized in deionized water, and protein and calcium were determined. In order to measure initially bound (intracellular) calcium, samples without added ATP were carried through the entire procedure. The intrinsic calcium is not removable by repeated washings with buffers (8). Analysis for calcium was in deproteinized solutions containing 0.5% trichloroacetic acid, 0.5% La(III), and 5 mM CsCl. A Perkin-Elmer (model 303) atomic absorption spectrophotometer was used.

**Filtration Method**—For kinetic studies tubes were incubated at 37°C in an incubation medium containing 45Ca and limited amounts of ATP. The reaction was terminated by filtering aliquots through 0.45 μm pore size Millipore filters with prefiltrers as outlined previously (4). Aliquots of the filtrate were counted in a Beckman LS-100 liquid scintillation system using Nari Solve VI. The calcium bound or released was computed from the difference between counts in the incubation medium and the filtrate, using appropriate controls not containing protein. Correction for exchange of 45Ca with intrinsic cold calcium was made.

**Calcium Exchange**—Exchange of intrinsic calcium with 45Ca added in the incubation medium was evaluated using the method and computation of Carvalho (10), as outlined by us previously (4). Analyses for calcium were done by atomic absorption spectroscopy on the pellets and supernatants after centrifugation. Radioactivity was determined on aliquots of the same solutions used for calcium analyses. From these data the specific activity (counts per min per nmol of calcium) of bound and free calcium was calculated. The calcium-activated ATPase could not be demonstrated at 25°C.

**ATPase Measurements**—ATPase activity was measured on filtrated aliquots after precipitation in 5% trichloroacetic acid. Liberation of inorganic phosphate was measured (11).

**Materials**—All chemicals were reagent grade. ATP (Sigma Chemical Co.) and sucrose, special enzyme grade (Schwarz-Mann), were used. ATP was freed of traces of calcium by treatment with Dowex 50-WX8-H+ as previously described (12). Oxytocin was obtained from Calbiochem. Prostaglandins were generously supplied by the Upjohn Co. Prostaglandin E1 (PGE1) and Fα (PGE2) were in aqueous solution. Prostaglandin Fα (PGE2) was dissolved in 30% alcohol. Controls of POP2 contained a comparable amount of alcohol.

**Computations and Statistics**—ATP-dependent calcium binding was computed as the difference in calcium in the presence and absence of ATP. All experiments were performed in duplicate. A minimum of six experiments were performed and averaged for statistical evaluation. The standard error of the mean was calculated.

1 The abbreviations used are: PG, prostaglandin; EGTA, ethylene glycol bis (β-aminoethyl ether) N,N'-tetraacetic acid; SR, sarcoplasmic reticulum.

**RESULTS**

ATP-dependent Calcium Binding—ATP-dependent calcium binding at 25°C and 37°C amounts to 16 and 24 nmol of calcium/mg of protein, respectively, at 8 min as shown in Fig. 1. The calcium present in the absence of ATP (intracellular calcium) is calcium in the preparations which cannot be removed by repeated washes with buffer. This amounted to 21.2 ± 1.1 nmol/mg of protein and has been subtracted in the data given in Fig. 1. At 0-4°C, no ATP-dependent calcium binding was detectable, and there was no release of calcium previously bound in the presence of ATP in periods up to 32 min. Oxalate is a known potentiatior of calcium accumulation in sarcoplasmic reticulum from other muscles. Addition of 1 mM potassium oxalate to the incubation medium resulted in a slightly increased calcium uptake, but the increase was not statistically significant at 8 min.

**ATPase**—ATPase activity is found in many tissue fractions. A calcium- and magnesium-activated ATPase is thought to be specific for a calcium-accumulating vesicular fraction derived from the sarcoplasmic reticulum. Calcium-activated ATPase activity was determined and the results are presented in Fig. 2. In the absence of calcium there is a small, but significant, decrease in ATP splitting at 37°C (at 4 and 8 min p < 0.05, at 16 min p < 0.005) suggesting a role for a calcium-stimulated ATPase in calcium binding. The calcium-activated ATPase could not be demonstrated at 25°C.

**Calcium Exchange**—We have reported that part of the intrinsic calcium exchanges with 45Ca added in the incubation medium (8). Since in smooth muscle intrinsic calcium is large relative to the ATP-dependent calcium binding, allowance has to be made for exchange of 45Ca by applying a correction when computing results in the 45Ca Millipore filtration method. We therefore re-evaluated the exchange of 45Ca with intrinsic calcium under the experimental conditions used in the present study. The experimental protocol for measuring the size of the exchangeable calcium pool is given in Table I.

The results show that at least half of the intrinsic calcium is exchangeable under all conditions tested. In the presence of ATP there is 6.8 nmol of calcium bound as deter-
 TABLE I

<table>
<thead>
<tr>
<th>Calcium in pellet</th>
<th>ATP-stimulated calcium binding</th>
<th>Calcium exchanged</th>
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<tbody>
<tr>
<td></td>
<td>nmol/mg protein</td>
<td>In pellet*</td>
</tr>
<tr>
<td>1. Complete medium</td>
<td>28.7 ± 1.7</td>
<td>6.8 ± 1.2</td>
</tr>
<tr>
<td>2. ATP omitted</td>
<td>21.0 ± 1.4</td>
<td>0</td>
</tr>
<tr>
<td>3. ATP omitted, 50 μg/ml PGE₂ added</td>
<td>22.7 ± 1.6</td>
<td>0</td>
</tr>
</tbody>
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* The difference between 1 and 2, ρ < 0.0005; between 1 and 3 ρ < 0.02; between 2 and 3 ρ < 0.10, i.e. not significant.

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 Corrections for volume changes were made in the calculation. Correction was also made for the exchangeable calcium, inasmuch as the $^{40}$Ca label was taken to represent the sum of the added calcium (20 mM) plus the exchangeable calcium which amounted to 11.9 nmol of Ca$^{2+}$/mg of protein in these experiments. The results graphed in Figs. 3 to 6 reflect the ATP-dependent calcium binding above and distinct from calcium exchange, i.e. the zero point is taken as zero net binding. In the absence of ATP the measured apparent calcium binding (removal of counts from the medium) is due to calcium exchange with the intrinsic calcium and to nonspecific binding to the microsomal protein. PGE was no effect on these.

Oxytocin, another uterine contractile agent previously found to inhibit ATP-dependent calcium accumulation (7), also caused a statistically significant calcium release from partially preloaded microsomal preparations (Fig. 5). In addition, at a concentration of 50 microunits/ml, oxytocin released some of the intrinsic calcium, as seen in Fig. 5.

The antibiotic ionophores X537A (Hoffman La Roche) and A23187 (Eli Lilly and Co.) are known to inhibit calcium binding and to release calcium from skeletal and cardiac muscle sarcoplasmic reticulum previously having accumulated calcium due to ATP stimulation (14, 15). In Fig. 6 we show that at appropriate concentrations these ionophores completely release previously accumulated calcium from uterine microsomal preparations. They have no effect on intrinsic calcium levels.

**DISCUSSION**

This study examined the calcium binding and transport properties of a vesicular microsomal fraction largely derived from the membranes of the sarcoplasmic reticulum of the myometrium. One would like to compare the calcium-binding properties with those of similar microsomal fractions from other smooth muscles. Comparisons are difficult, however, because ATP-dependent calcium binding depends on experimental conditions, particularly it increases with the calcium concentration in the medium (16). At lower calcium concentration than used by others our calcium binding appears to be higher than that reported in other smooth muscles such as guinea pig ileum (17), bovine aorta (16, 18), and rat aorta (19).

Improvements in preparative procedures have led to greater ATP-dependent calcium binding and lower yields, compared with our previous studies (4, 8). Calcium accumulation per kg of tissue has almost doubled over our previous results and is now 2074 nmol/kg of tissue in 1 min. In other muscles, calcium uptake in SR fractions is coupled to a calcium- and magnesium-activated ATPase. We have demonstrated a calcium- and magnesium-activated ATPase in the uterine microsomal fraction. Since this ATPase is only a small fraction of the total ATPase, and the systematic error is relatively high for the expected small differences, it appears that the calcium- and magnesium-activated ATPase can be demonstrated convincingly only when relatively large amounts of ATP are split, i.e. at the 16 min point. Accordingly, at the lower level of ATP splitting, at 25°C, it could not be detected. The temperature sensitivity of the various ATPases is unknown. It is not possible to calculate precise Ca/P ratios for initial velocities, which has been done for calcium uptake (in the presence of oxalate) in other muscles, because we cannot detect significant CaATPase activity until the reaction has proceeded for 16 min. At that point the net calcium accumulation has slowed considerably. The simplest interpretation for our results is that there is a limited capacity for calcium binding while the CaATPase remains active. Nevertheless, the results suggest that the ATP splitting could well provide the energy for calcium transport. This calcium transport is temperature-dependent and thus compatible with an enzymatic reaction.

Since the intrinsic calcium is almost as high as the ATP-dependent calcium binding in the microsomal fraction containing sarcoplasmic reticulum, it was of interest to determine whether calcium exchange was a factor obscuring results of calcium binding studies. Our finding that more than half of the intrinsic calcium exchanges with calcium added in the incubation medium, warrants appropriate corrections to

**FIG. 5.** Effect of oxytocin on calcium release. Incubation medium as in Fig. 4. Oxytocin added at arrow, 50 microunits/ml of incubation medium.

**FIG. 6.** Effect of ionophores on calcium release. Incubation medium as in Fig. 4. Ionophores X537A and A23187 were added at arrow; 20 μM X537A, 0.1 μM A23187.
be made in all computations based on experiments using the 44Ca Millipore filtration method. The magnitude of this correction is a function of the microsomal fraction used and may vary with the state of purification and with the preparative procedure (20). Thus, in our older preparations, substantially less calcium was found to be exchangeable (6, 20).

Our results show two pools of intrinsic calcium, one exchangeable and one not exchangeable. The next question is, which pool does the calcium bound under ATP stimulation join?

If we assume that ATP-dependent calcium binding is much faster than exchange, this bound calcium must be completely exchangeable to explain our experimental result, namely that the increase in exchangeable calcium is equal to the amount of calcium bound. However, we know that calcium binding is not instantaneous. Furthermore, our results from kinetic studies show calcium exchange virtually complete in 0.5 min, whereas ATP-dependent calcium binding continues. If exchange is slower than calcium binding, it is unlikely that the system would have reached equilibrium at the time of sampling. If it had not reached equilibrium, we should have obtained a significantly smaller amount of calcium exchangeable than calcium bound. But our results showed the two figures to be equal.

An alternative explanation is that exchange is much faster than ATP-dependent calcium binding. In this case, exchange occurs with the intrinsic calcium pool. The calcium in the medium is then taken up in the appropriate ratio. If this mechanism applies, we would not know whether this calcium remains freely exchangeable after it has been sequestered. Intermediate conditions between these two hypotheses may exist. Thus our results do not allow us to conclude whether this calcium remains in the exchangeable pool or how it is compartmentalized. Further experiments are in progress to elucidate this point.

PGE2 and PGF2α are known uterine contractile agents (21). PGF6α is a physiologically inactive analogue. We have previously shown their overall inhibition of calcium accumulation to be consistent with their biological activity on the uterus. Calcium release was now found with PGE2, to a lesser degree than with ATP-stimulation, facilitating release of calcium from the intrinsic calcium pool. Thus, the mode of action of oxytocin may differ from that of prostaglandins and ionophores.

In our system, the effect of prostaglandins or of the ionophores cannot be due to changing enzyme activation by binding and thereby removing activator calcium from solution, because the release of calcium is not enzyme-activated. Furthermore, although prostaglandins, like ionophores, form calcium complexes, these are weak calcium complexes. In our laboratory, the association constant of PGE2 was found to be 326 ± 18.1,2 while PGE4 forms no calcium complex. This association constant is not high enough to remove significant amounts of calcium from solution at the low PG concentration used. Our results of this investigation show that a more increase in the calcium concentration gradient does not in itself cause release of calcium.

Thus, it appears that the prostaglandins in a manner similar to the ionophores, facilitate release of calcium from the microsomal vesicles down a transmembrane gradient. Results of experiments in progress in this laboratory have shown a weak ionophoretic action for prostaglandins in an in vitro model system as used for antibiotic ionophores (25). The results of these experiments will be published elsewhere. It is suggested that the uterine-contracting prostaglandins can function as calcium ionophores. The extent to which the ionophoretic action plays a significant role in the physiologic process remains to be elucidated.

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REFERENCES

Effects of prostaglandins and oxytocin on calcium release from a uterine microsomal fraction.
M E Carsten and J D Miller


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