Oxytocin Inhibition of \( (Ca^{2+} + Mg^{2+}) \)-ATPase Activity in Rat Myometrial Plasma Membranes*

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Enriched plasma membranes from uterine smooth muscle of estrogen-treated rats were prepared by discontinuous sucrose gradient centrifugation. This specific fraction contained oxytocin receptors and oxytocin-inhibited calcium-stimulated and magnesium-dependent adenosine-triphosphatase \( (Ca^{2+} + Mg^{2+}) \)-ATPase activity. Membranes from myometria of rats treated with progesterone lacked both basal and oxytocin-inhibited \( (Ca^{2+} + Mg^{2+}) \)-ATPase activities. The oxytocin-inhibited enzyme also was found in plasma membranes from rat adipocytes, which are oxytocin target cells, but not from a nontarget tissue like dorsal smooth muscle. Half-maximal inhibition of \( (Ca^{2+} + Mg^{2+}) \)-ATPase activity in myometrial membranes occurred with about 1 nM oxytocin, corresponding to the apparent \( K_d \) of oxytocin-receptor interaction. Several synthetic oxytocin analogues inhibited myometrial \( (Ca^{2+} + Mg^{2+}) \)-ATPase activity in proportion to their ability to stimulate uterine contractions.

Oxytocin-inhibited \( (Ca^{2+} + Mg^{2+}) \)-ATPase in the myometrium had a \( V_{max} \) of about 0.2 amol/min/mg of protein, a \( K_M \) of about 50 nM Mg-ATP, and a Hill coefficient of 1.85. Maximal inhibition by oxytocin occurred with the lowest \([Ca^{2+}]\) tested, 60 nM. Oxytocin only partially inhibited the enzyme at \([Ca^{2+}]\) of 5 \( \mu \)M or less, but complete inhibition was seen at higher \([Ca^{2+}]\). These results indicate that \( (Ca^{2+} + Mg^{2+}) \)-ATP activity in the myometrium is composed of more than one enzyme, one of which is inhibited by oxytocin. Oxytocin-inhibited \( (Ca^{2+} + Mg^{2+}) \)-ATPase was not affected by \([Na^+] \) or \([K^+] \) ranging from 10 to 200 mM, suggesting that counter-ion transport is not necessary for activity. Oxytocin action may be regulated by calmodulin because 2 \( \mu \)M trifluoperazine, which inhibited the effect of oxytocin on \( (Ca^{2+} + Mg^{2+}) \)-ATPase activity. These studies provide a basis for postulating a mechanism of induction of uterine contraction by enzymatic regulation of intracellular calcium concentrations in response to oxytocin-receptor interaction.

Despite extensive studies on the characterization of oxytocin receptors in the rat myometrium (1), little is known of the molecular actions of oxytocin beyond its interaction with receptor sites. It is likely that \( Ca^{2+} \) is necessary for coupling excitation and contraction of uterine smooth muscle (2), but the origin of \( Ca^{2+} \) activating uterine contractions is unknown. Because the myometrial cell membrane maintains a large electrochemical gradient for ionized calcium between the cytoplasm and the extracellular space, the sarcolemma may be an important site for the regulation of intracellular \([Ca^{2+}] \) and therefore for the action of oxytocin. Akerman and Wikstrom (3) reported that \( (Ca^{2+} + Mg^{2+}) \)-ATPase, but not \( (Ca^{2+}) \)-ATPase, in the myometrium of estrogen-treated rabbits was inhibited in vitro by oxytocin. Although these results were preliminary, they implied that the enzyme may catalyze ATP-dependent \( Ca^{2+} \) extrusion from uterine smooth muscle cells in the manner that has been described for erythrocytes (4), nerve (5), liver (6), smooth muscle (7), and adipose cells (8). In the present study, we have sought to characterize the \( (Ca^{2+} + Mg^{2+}) \)-ATPase in myometrium from estrogen-treated rats and to study the inhibitory effects of oxytocin.

EXPERIMENTAL PROCEDURES

Materials—Rats, 2 months old, were purchased from ARS Sprague-Dawley, Madison, Wisconsin. \([\alpha-32P]ATP\) (30 Ci/mmol) was purchased from New England Nuclear. Oxytocin and lysine vasopressin were gifts from Sandoz Ltd., East Hanover, NJ. The oxytocin analogues HO\[Thr^4\]oxytocin, arginine-vasopressin and [d(CH₂)₅Thr⁴]oxytocin were donated by Dr. Maurice Manning of this department. [2-O-Me]Oxytocin was a gift from Dr. Karel Jost, Czechoslovak Academy of Science, Institute of Organic Chemistry and Biochemistry, Prague. Bradykinin triacetate was purchased from Sigma. The divalent cation ionophore, A23187, was a gift from Eli Lilly. Trifluoperazine was from Smith, Kline and French Laboratories. [Tyrosine-3H]oxytocin (34 Ci/mmol) was a gift from Dr. Luis Branda, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada. This material was repurified before use (9). Calmodulin was a gift from Dr. Erwin Reimann of this department.

Preparation of \( (Ca^{2+} + Mg^{2+}) \)-ATPase—Rats were injected subcutaneously with 5 mg of diethylstilbestrol in 0.3 ml of sesame oil for 2 days and were killed by cervical dislocation on the third day. The uterine horns were excised, trimmed of connective tissue, fat, and endometrium. The myometrium was minced with a McIlwain tissue chopper and homogenized at 4 °C in 5 volumes of 100 mM KCl, 5 mM MgCl₂, 50 mM Tris-HCl, pH 7.2. The homogenate was centrifuged 20,000 \( \times \) g for 10 min to remove nuclei, mitochondria, and fibrous material. The supernatant was removed and centrifuged 160,000 \( \times \) g for 30 min. The pellet was briefly homogenized in 10% sucrose in homogenization buffer and the suspension was layered onto an equal volume of 28% (w/w) sucrose in homogenization buffer. The samples were centrifuged 31,000 rpm in a SW-50 rotor for 1 h. The membrane fraction at the 10/28% sucrose interface was withdrawn with a pipette and diluted with assay buffer containing no calcium (see below). The samples were then centrifuged at 160,000 \( \times \) g for 30 min and the pellets, essentially free of sucrose, were resuspended in homogenization buffer. An aliquot was taken for the

1 The abbreviations used are: (Ca²⁺ + Mg²⁺)-ATPase, calcium-stimulated and magnesium-dependent adenosine triphosphatase; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HO[Thr⁴]oxytocin, [1-(6-2-hydroxy-5-mercaptopropionoic acid), 4-threonine]oxytocin; [d(CH₂)₅Thr⁴]oxytocin, (1-(β-mercaptop-β,β-cyclophosphamethylenepropionic acid), 4-threonine]oxytocin; [2-O-Me]oxytocin, (2-O-methyltyrosine]oxytocin; TFP, trifluoperazine.
determination of protein by the method of Lowry et al. (10), with serum albumin as standard.

Epithyal fat pads were taken from 8 male rats, 150-200 g, that were injected with 5 μg of diethylstilbestrol for 2 days. A section of duodenum comparable in size to the uterus, was taken from estrogen-treated rats. The mucous lining was scraped off in a manner similar to removal of the uterine endometrium. The membrane fractions of homogenates of myometrium, gut, and fat pads were prepared identically.

In confirmation of the report by Åkerman and Wikström (3), we found that (Ca$^{2+}$ + Mg$^{2+}$)-ATPase and/or oxytocin-inhibited ATPase activities were unstable (storage at 4°C or -80°C overnight resulted in the loss of activity). Also as reported (3), we found that oxytocin was consistently inhibitory but the extent of inhibition was quite variable. The data shown in the figures are representative of a number of experiments on separate uterine preparations.

(Ca$^{2+}$ + Mg$^{2+}$)-ATPase Assay—Membrane fractions were assayed in 50 mM Tris-HCl, 20 mM HEPES, pH 7.0, containing 5 mM NaN$_3$, 1 mM MgCl$_2$, 2 mM Mg-ATP (unless otherwise indicated), and 30 μM free calcium (unless otherwise indicated). Based on the observations of Wuytack and Casteels (11), the incubation mix (500 μl) also contained 0.1 μM isoprenaline A23187. In preliminary studies, A23187 was found to stimulate (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity without affecting its inhibition by oxytocin. Oxytocin was used at 40 millionths/ml (90 nM), unless otherwise noted. Calcium-stimulated activity was determined by subtracting values obtained with 0.5 or 1 mM EGTA alone from those obtained with Ca-EGTA buffers. The samples were preincubated for 10 min at 37°C in the absence of ATP. The reaction was initiated by the addition of ATP and terminated by the addition of an equal volume of 10% (w/v) trichloroacetic acid. After centrifugation, the concentration of inorganic phosphate in the supernatant was determined according to King (12). Several time points were taken to ensure that the rates were linear. Initial velocities, determined with 10% or less of the ATP hydrolyzed, were directly proportional to the amount of protein added. ATP hydrolysis was measured also by the isotopic assay of Seals et al. (13), with a total Mg-ATP concentration of 0.5 mM.

Calcium Concentration—Total calcium concentrations were determined by atomic absorption spectrophotometry with internal standards. Free calcium concentrations were calculated from EGTA/calcium ratios as described by Schatzmann (14). The total EGTA concentration was kept at 200 μM and increasing concentrations of calcium were used. The concentration of Mg$^{2+}$ in excess of Mg-ATP was kept at 1 mM to prevent Ca-ATP formation.

Sucrose Gradient Fractionation—Microsomal fractions of the myometrial homogenates were resuspended in 10% (w/w) sucrose in homogenization buffer and 3 ml were applied to a discontinuous sucrose gradient composed of the following percentages (w/w) of sucrose in homogenization buffer: 3 ml of 50%, and 3.5 ml each of 42, 35, and 28%. The tubes were centrifuged at 25,000 rpm in a SW28 rotor for 2 h at 4°C. Fractions comprising each concentration of sucrose and the interfaces between sucrose concentrations were collected and assayed for the following activities: A, ATPase minus oxytocin (Δ); ATPase plus oxytocin (Δ). The difference between these activities is expressed as oxytocin-inhibited ATPase (Δ). B, 5'-nucleotidase (Δ); [3H]oxytocin binding (Δ); oxytocin-inhibited ATPase (Δ). C, succinate dehydrogenase (Δ); rotenone-inhibitable NADH cytochrome c reductase (Δ); D, RNA (Δ); acid phosphatase (Δ). Myometria from 10 rats were pooled for preparation of the microsomal fraction. The results, shown for one preparation, are representative of assays of three separate microsomal fractions.

The distributions of RNA and acid phosphatase, a lysosomal marker, also were distinct from that of the oxytocin-inhibited ATPase.

Oxytocin-inhibited (Ca$^{2+}$ + Mg$^{2+}$)-ATPase

found that the greatest specific activity of oxytocin-inhibited ATPase was associated with the microsomal fraction of the myometrium of estrogen-treated rats (data not shown). Further fractionation of this material by discontinuous sucrose gradient centrifugation resulted in the cofractionation of oxytocin-inhibited ATPase with oxytocin binding sites and 5'-nucleotidase, both markers for plasma membranes (Fig. 1). The distribution pattern of oxytocin-inhibited ATPase was distinct from those of mitochondrial markers, such as succinate dehydrogenase and rotenone-insensitive NADH cytochrome c reductase (mitochondrial outer membrane) (Fig. 1). The distributions of RNA and acid phosphatase, a lysosomal marker, also were distinct from that of the oxytocin-inhibited ATPase.
ATPase. The results indicate that oxytocin-sensitive ATPase is localized on the plasma membrane and that the effects of oxytocin may be mediated by [3H]oxytocin binding sites previously characterized as oxytocin receptors (1, 18-22).

Although the bulk of mitochondria were removed by differential centrifugation, the gradient profiles indicated the presence of mitochondrial contamination in the enriched plasma membrane fraction (10/28% sucrose interface). Because mitochondria contain ATPase activity that might contribute to basal ATPase levels, we sought to inhibit selectively this activity with sodium azide. Inclusion of 5 mM azide in the assay medium resulted in a 48% reduction in basal ATPase activity (Table I). However, there was no effect of azide on the per cent of oxytocin-inhibited ATPase activity relative to the new basal levels (Table I). The presence of azide in the mitochondrial-rich fraction (28/35% sucrose interface) reduced basal activity 88% and obliterated oxytocin-inhibited ATPase (Table I). Although azide may not be an entirely specific inhibitor of mitochondrial ATPase, we included it in all subsequent assays to minimize the contribution of mitochondrial ATPase to baseline levels. Henceforth, the 10/28% sucrose gradient fraction was used for all assays.

Effect of Estrogen and Progesterone on Oxytocin-inhibited ATPase Activity—The preceding studies were carried out on rats injected with 5 μg of dihydrotestosterone for each of 2 days before killing. With this regimen, there was a 44% reduction of basal ATPase activity in the presence of 90 nM oxytocin (Table II). The same extent of inhibition occurred in the presence of 0.5 mM EGTA alone or in combination with oxytocin (Table II). Because there was no additive inhibition of ATPase by EGTA and oxytocin, these results suggest that oxytocin inhibits a (Ca2+ + Mg2+)-ATPase. Treatment of rats with 5 mg of progesterone in addition to dihydrotestosterone resulted in the loss of inhibition of ATPase activity by EGTA, oxytocin, and EGTA and oxytocin combined (Table II). These findings suggest that progesterone is required for both (Ca2+ + Mg2+)-ATPase and oxytocin-inhibited activities.

Tissue Specificity of Oxytocin-inhibited ATPase—Both duodenal smooth muscle and adipose tissue membrane preparations had (Ca2+ + Mg2+)-ATPase activities (Table III). Oxytocin had no effect on the ATPase activity of duodenum but suppressed ATPase in adipose tissue to the same extent as EGTA (Table III). The fraction of oxytocin-inhibited ATPase in fat tissue was about 0.15 whereas it was about 0.43 in the myometrium (Table III). These results are consistent with the fact that myometrial and fat cells are both targets for oxytocin, whereas intestinal smooth muscle is not. There was a small but consistent inhibition by oxytocin of ATPase activity in the plasma membrane fraction of homogenates of lactating rat mammary gland, another oxytocin target tissue.

Peptide Specificity of Myometrial (Ca2+ + Mg2+)-ATPase

Inhibition—The concentrations of oxytocin giving half-maximal and maximal inhibition of ATPase activity were about 1 and 10 nM, respectively (Fig. 2). ATPase activity also was inhibited by increasing concentrations of oxytocin analogues and bradykinin, a nonpeptide that causes myometrial contraction (Fig. 2). For most analogues the regressions were parallel, indicating a common set of receptor sites, as has been shown in studies on the inhibition of [3H]oxytocin binding to uterine and mammary cell membranes by oxytocin analogues (19-22). The inhibitory potencies of each peptide relative to oxytocin were: oxytocin, 1; HO[Thr4]oxytocin, 2.6; bradykinin, 0.17; [Arg5]vasopressin and [Lys7]vasopressin, 0.1; [d(CH2)5Thr6]oxytocin, 0.65; and [2-O-Me]oxytocin, 5 × 10-4.

Oxytocin-inhibited ATPase—Oxytocin-inhibited ATPase in the enriched plasma membrane fraction of rat myometrium had a Vmax of approximately 0.2 pmol/min/mg of protein in most preparations (Fig. 3). In some preparations, values as high as 0.7 pmol/min/mg were found. The K0.5 for ATP was about 50 μM. This system exhibited positive cooperativity with respect to the concentration of ATP, as indicated by a bell-shaped Eadie Hofstee plot (Fig. 3, inset). A Hill plot of the data gave a slope of 1.85 (Fig. 4).

**Table II**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxytocin (90 nM)</th>
<th>EGTA (0.5 mM)</th>
<th>ATPase activity (μmol P_i/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen (5 μg/day × 2)</td>
<td>− −</td>
<td>1.65 ± 0.09 (n = 6)</td>
<td></td>
</tr>
<tr>
<td>+ +</td>
<td>0.92 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ +</td>
<td>0.96 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ +</td>
<td>0.99 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen (5 μg/day × 2) plus progesterone (5 mg/day × 2)</td>
<td>− −</td>
<td>1.56 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>+ +</td>
<td>1.48 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ +</td>
<td>1.56 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ +</td>
<td>1.41 ± 0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>Sample</th>
<th>ATPase activity (μmol P_i/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myometrium*</td>
<td>1.51 ± 0.06 (n = 6)</td>
</tr>
<tr>
<td>+ Oxytocin</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>+ EGTA</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>+ Oxytocin + EGTA</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>Duodenum+</td>
<td>1.34 ± 0.06 (n = 6)</td>
</tr>
<tr>
<td>+ Oxytocin</td>
<td>1.29 ± 0.04</td>
</tr>
<tr>
<td>+ EGTA</td>
<td>0.78 ± 0.11</td>
</tr>
<tr>
<td>+ Oxytocin + EGTA</td>
<td>0.78 ± 0.11</td>
</tr>
<tr>
<td>Fat pad</td>
<td>0.52 ± 0.02 (n = 2)</td>
</tr>
<tr>
<td>+ Oxytocin</td>
<td>0.44 ± 0.0</td>
</tr>
<tr>
<td>+ EGTA</td>
<td>0.43 ± 0.0</td>
</tr>
<tr>
<td>+ Oxytocin + EGTA</td>
<td>0.43 ± 0.0</td>
</tr>
</tbody>
</table>

* Myometrium and duodenum were taken from the same animals.
+ 90 nM oxytocin.
+ 0.5 mM EGTA.

2 M. S. Soloff and P. Sweet, unpublished data.
Oxytocin-inhibited (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase

With the lowest concentration of free calcium tested, 60 nM (Fig. 7). Between 0.1 and 2 μM Ca\(^{2+}\), the activity was reduced to a lower, generally constant level and then fell further at concentrations between 2 and 25 μM. Oxytocin completely inhibited total (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase activity at concentrations of free Ca\(^{2+}\) of 10 μM or greater (Fig. 7). Partial inhibition was observed at lower Ca\(^{2+}\) concentrations. Oxytocin-inhibitable (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase activity was maximal at 0.06 μM calcium but a constant level of inhibition was observed over the Ca\(^{2+}\) concentration range of 0.1 to 25 μM (Fig. 7, inset).

Lack of Effect of Na\(^{+}\), K\(^{+}\), or Ouabain on (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase-To investigate whether counter-ion transport is necessary for (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase activity, we measured the effects of increasing concentrations of Na\(^{+}\) and K\(^{+}\) on basal and oxytocin-inhibited ATPase. There was no effect of 0, 10, 25, 50, 75, 150, or 200 mM Na\(^{+}\) or K\(^{+}\) on either ATPase activity (data not shown). Ouabain, 1 mM, also had no effect on either ATPase. Mn\(^{2+}\) or Co\(^{2+}\), 5 mM, both reduced basal ATPase activity.

Calcium Dependency of (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase Activity and Oxytocin-inhibited ATPase Activities—Maximal stimulation of the EGTA suppressible ATPase activity occurred

![Graph](http://www.jbc.org/DownloadedFrom)

Fig. 3. Effect of increasing Mg-ATP concentration on ATPase activity in the absence (O) and presence (●) of 90 nM oxytocin. The difference in ATPase activity (Δ) is referred to as oxytocin-inhibited ATPase. Oxytocin-inhibited ATPase activity also is shown as an Eadie-Hofstee plot (inset). The results shown for one experiment are typical of four separate assays on different preparations, in which oxytocin inhibition of ATPase activity ranged between 20 and 50% of basal activity.

![Graph](http://www.jbc.org/DownloadedFrom)

Fig. 4. Hill plot of oxytocin-inhibited ATPase activity depicted in Fig. 3. The line, representing the best fitting form of the Hill equation, gave a coefficient of 1.85.

5). At higher ATP concentrations, 0.5 and 1 mM, an additional calcium-sensitive ATPase was seen with an apparent V\(_{\text{max}}\) of about 0.35 μmol/min/mg (not shown). The K\(_{\text{m}}\) of the higher affinity component was about 15 μM ATP.

As indicated by the nonlinear Eadie-Hofstee plots of (Mg\(^{2+}\))-ATPase and (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase activities (Fig. 5, inset), the ATPases appear to demonstrate negative cooperativity. Both enzyme systems had Hill coefficients of less than 1 (Fig. 6). In addition, (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase activity demonstrated positive cooperativity at the lower concentrations of ATP, 10 and 12.5 μM (Fig. 6). Extrapolation of the linear portion of the Eadie-Hofstee plot of (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase suggests that the high affinity component had a K\(_{\text{m}}\) of less than 2 μM ATP. Because these enzyme systems are not pure, it is difficult to make more accurate estimations.

Calcium Dependency of (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase Activity and Oxytocin-inhibited ATPase Activities—Maximal stimulation of the EGTA suppressible ATPase activity occurred

![Graph](http://www.jbc.org/DownloadedFrom)

Fig. 5. Effect of increasing Mg-ATP concentration on ATPase activity in the absence (O) and presence (●) of 500 mM EGTA. Each point is the mean ± S.E. of four separate myometrial preparations in quadruplicate. The difference in ATPase activity (Δ) is referred to as (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase. Both activities also are shown as Eadie-Hofstee plots (inset).

![Graph](http://www.jbc.org/DownloadedFrom)

Fig. 6. Hill plots of (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase and (Mg\(^{2+}\))-ATPase activities in myometrial plasma membrane fractions depicted in Fig. 5. The Hill coefficient for the (Mg\(^{2+}\))-ATPase was 0.7. (Ca\(^{2+}\) + Mg\(^{2+}\))-ATPase activities were comprised of at least two components, one with a Hill coefficient > 1 and the other with n = 0.24.
Activity-TFP, suppress this basal activity (Fig. 8A). In the presence of 1 mM compared to activity with no TFP, and oxytocin did not inhibit almost completely all ATPase activities (Fig. 8A). Selectivity block calmodulin regulated activities (23, 24), in absence of Mg-ATP concentration. Oxytocin-inhibited (Ca2+ + Mg2+)-ATPase activity (inset) measured by subtracting (Ca2+ + Mg2+)-ATPase activity measured in the presence of 90 nM oxytocin from EGTA-inhibited ATPase activity (minus oxytocin). Each point is the mean of quadruplicates of a single enzyme preparation. Comparable results were obtained with two other preparations.

ATPase by about 50% or more but had no specific effect on the fraction of oxytocin-inhibited ATPase. The divergent cation ionophore, A23187, increased by as much as twice the relative inhibition measured in the presence of 500 mM EGTA from total activity (1 mM excess Mg2+ over Mg-ATP concentration). Oxytocin-inhibited (Ca2+ + Mg2+)-ATPase activity was determined by subtracting (Ca2+ + Mg2+)-ATPase activity measured in the presence of 90 nM oxytocin from EGTA-inhibited ATPase activity (minus oxytocin). Each point is the mean of quadruplicates of a single enzyme preparation. Comparable results were obtained with two other preparations.

Effect of Trifluoperazine on Oxytocin-inhibited ATPase Activity—TFP, 200 μM, which has been used by others to selectivity block calmodulin regulated activities (23, 24), inhibited almost completely all ATPase activities (Fig. 8A). At a lower concentration, 2 μM TFP reduced basal ATPase (as compared to activity with no TFP), and oxytocin did not suppress this basal activity (Fig. 8A). In the presence of 1 mM EGTA, 2 μM TFP was ineffective. Similar results were obtained with 20 μM TFP, except that the suppression of basal ATPase activity was considerably greater (Fig. 8A).

In two out of three experiments with separate uterine preparations, exposure of homogenates to 0.1 mM EDTA, in absence of divalent cations in the medium, resulted in a loss of oxytocin-inhibited ATPase activity (Fig. 8B). These findings, in conjunction with the TFP results, suggest that oxytocin-inhibited ATPase activity is a calmodulin-regulated (Ca2+ + Mg2+)-ATPase. However, addition of calmodulin to the assay medium had no effect on either (Ca2+ + Mg2+)-ATPase or oxytocin-inhibited ATPase activities (data not shown).

DISCUSSION

Although Ca2+ forms an integral part of the excitation-contraction sequence in myometrial smooth muscle cells, we do not know the location of the activator Ca2+, the factors involved in its mobilization, or the path of its movement. The possible mechanisms leading to increased myoplasmic [Ca2+] include increased Ca2+ release from storage sites on the cell surface, on the sarcoplasmic reticulum and/or mitochondria, acceleration of Ca2+ movement from the extracellular space by a gate mechanism, or inhibition of calcium pumps involved in the extrusion of myoplasmic Ca2+. A calcium pump associated with cell membranes serves to maintain cytoplasmic concentrations of Ca2+ at a level more than 10,000 that of the extracellular free calcium concentrations (25, 26). This gradient is maintained in certain cell types by the utilization of ATP in reactions catalyzed by (Ca2+ + Mg2+)-ATPases (27-31). The inhibition by oxytocin of myometrial (Ca2+ + Mg2+)-ATPase activity, resulting in increased myoplasmic [Ca2+], may be the mechanism by which uterine contractions are induced by the hormone. Consistent with this postulate, oxytocin-inhibited (Ca2+ + Mg2+)-ATPase activity was associated with the plasma membrane fraction and not with endoplasmic reticulum, which has been shown in other tissues to possess high affinity (Ca2+ + Mg2+)-ATPase activity (32, 33).

Several lines of evidence indicate that oxytocin-inhibited ATPase and a (Ca2+ + Mg2+)-ATPase are one and the same enzyme. Both activities required Ca2+ and were inhibited by trifluoperazine. The effects of oxytocin and EGTA were not additive when [Ca2+] was greater than 10 μM, suggesting that the same enzyme system was inhibited by both agents. The two enzyme systems exhibited similar dose-response relationships with [Ca2+], although with 5 μM Ca2+ or less, (Ca2+ + Mg2+)-ATPase activity was not completely inhibited by oxytocin (Fig. 6). The apparent K0.5 for [Ca2+] in both systems was less than the lowest [Ca2+] studied, 0.06 μM. Because maximal stimulation was obtained at 0.06 μM Ca2+, it was not possible to determine accurately the K0.5 for Ca2+. (Ca2+ + Mg2+)-ATPase activities showed complex kinetics with increasing concentrations of Mg-ATP. Both negative and positive cooperativity were seen with respect to [Mg-ATP]. Oxytocin-inhibited ATPase activity, however, demonstrated only positive cooperativity. These results, taken together with the Ca2+-dose response results, suggest that there is more than one (Ca2+ + Mg2+)-ATPase, one of which appears to be inhibited by oxytocin.

Oxytocin-inhibited ATPase activities were present only in cell membrane fractions from tissues possessing oxytocin receptors (1, 18, 25, 38). (Ca2+ + Mg2+)-ATPase activity in the duodenum (39) was unaffected by oxytocin, consistent with the absence of any known effect of oxytocin action in the gut. The myometrial (Ca2+ + Mg2+)-oxytocin-inhibited ATPase appears to be similar to the enzyme in isolated adipocyte membranes (8). The fat cell enzyme requires Mg, has a K0.5
The specific inhibitory action of oxytocin on (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity provides a working hypothesis for the molecular action of oxytocin resulting in contraction of myometrial cells. Further studies will be required to establish a relationship between oxytocin-inhibited (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity and a calcium extrusion pump.

**Acknowledgments**—We thank Dr. Erwin Reimann for helpful discussions and Beverly Lockwood for secretarial assistance.

**REFERENCES**


Oxytocin-inhibited (Ca$^{2+}$ + Mg$^{2+}$)-ATPase

The concentration of oxytocin giving half-maximal inhibition of myometrial (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity was about 1 nm, corresponding to the apparent $K_i$ of oxytocin-receptor interaction (21). The results obtained with oxytocin analogues support the conclusion that oxytocin inhibition of ATPase activity is receptor-mediated. These analogues inhibited myometrial (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity in the same rank order as their potencies in stimulating uterine contractions. For example, HO[Thr$^3$]oxotocin has about twice the uterotonic potency (rat uterus) of oxytocin in the presence of 0.5 mM Mg$^{2+}$ (40), and was 2.6 times more potent than oxytocin in inhibiting myometrial (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity. The oxytocin antagonists (d[CH$_2$]$_3$Thr$^3$]oxotocin (41) and [2-O-Me$^3$]oxotocin (42) are weak uterotonic agonists, and exhibited low inhibition of (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity relative to oxytocin. The vasopressins also inhibited ATPase in proportion to their oxytocic potencies (43). Bradykinin, a nonapeptide unrelated to oxytocin but with about 25% of the oxytocic potency (44, 45), had 17% the potency of oxytocin in inhibiting myometrial ATPase. These results suggest that uterine contractions are initiated by occupancy of different oxytocin and bradykinin receptors that operate through a common pathway of the inhibition of (Ca$^{2+}$ + Mg$^{2+}$)-ATPase.

Estrogen treatment causes a marked rise in the concentration of myometrial oxytocin receptors (46, 47). We considered the possibility that the inhibitory action of oxytocin on the estrogen-dominated uterus was regulated solely at the receptor level. However, treatment of intact rats with progesterone, an estrogen antagonist that reduces the concentration of myometrial oxytocin receptors (47), resulted in the suppression of EGTA-inhibited ATPase activity. It is likely, therefore, that estrogen is required for increases in both oxytocin receptors and (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity in the myometrium.

We are not certain whether myometrial (Ca$^{2+}$ + Mg$^{2+}$)-ATP activity is regulated by calmodulin, as has been shown in erythrocytes (48-50). The addition of 200 µM trifluoperazine, an antipsychotic agent that selectively binds to calmodulin in a Ca$^{2+}$-dependent fashion (28), completely inhibited both (Mg$^{2+}$)-ATPase and (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activities. However, 2 µM trifluoperazine selectively inhibited only (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity. Removal of free Ca$^{2+}$ by the addition of EGTA reversed the inhibitory activity of TFP. These results are consistent with the calcium dependency of TFP binding to calmodulin (23). The loss of (Ca$^{2+}$ + Mg$^{2+}$)-ATPase activity by pretreatment of myometrial membranes with EDTA also is consistent with a calmodulin-mediated enzyme. However, these studies do not rule out the requirement of other protein activators that are dependent upon divalent cations (51).
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37. Thompson, K. E., Freychet, P., and Roth, J. (1973) Endocrinology 91, 1199-1205
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