Oxytocin increases the [Ca\textsuperscript{2+}]\textsubscript{i} sensitivity of human myometrium during the falling phase of phasic contractions

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Oxytocin increases the [Ca\textsuperscript{2+}]\textsubscript{i} sensitivity of human myometrium during the falling phase of phasic contractions. Am. J. Physiol. 276 (Endocrin. Metab. 39): E345–E351, 1999.—Oxytocin is commonly used to induce or augment labor, but its mode of action is uncertain. To address this issue, isometric tension and the intracellular free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) were simultaneously recorded from isolated strips of pregnant human myometrium loaded with fura 2. The changes in [Ca\textsuperscript{2+}]\textsubscript{i} and tension during phasic contractions were indistinguishable in myometrium taken before or after the onset of labor, enabling samples to be pooled. Oxytocin (10 nM) had no effect on basal [Ca\textsuperscript{2+}]\textsubscript{i} or tension, but it increased both the [Ca\textsuperscript{2+}]\textsubscript{i} and the tension recorded during phasic contractions. Analysis of the [Ca\textsuperscript{2+}]\textsubscript{i}-tension relationship revealed that during the falling (relaxation) phase of the contractile response, oxytocin increased the tension recorded at each [Ca\textsuperscript{2+}]\textsubscript{i}. By manipulating extracellular Ca\textsuperscript{2+} during phasic contractions, it was possible to ensure that the [Ca\textsuperscript{2+}]\textsubscript{i} signals were similar in the presence and absence of oxytocin, yet oxytocin still improved the [Ca\textsuperscript{2+}]\textsubscript{i}-tension relationship. We conclude that 10 nM oxytocin increases the [Ca\textsuperscript{2+}]\textsubscript{i} sensitivity of the contractile proteins only after a contraction has begun, possibly by causing inhibition of myosin light chain phosphatase.

smooth muscle; signal transduction; labor; pregnancy; uterus

OXYTOCIN INCREASES the force and duration of myometrial contractions and is used clinically for induction and augmentation of labor. Whether endogenous oxytocin is involved in the onset and progression of normal human labor is controversial (27). Neither the maternal nor fetal plasma oxytocin concentrations have been conclusively demonstrated to increase during labor (27). However, the sensitivity of the uterus to oxytocin increases during pregnancy (2), and oxytocin antagonists reduce the frequency of spontaneous contractions (5).

Oxytocin interacts with a member of the family of receptors that couple to G proteins and thereby stimulates, via G\textsubscript{q/11} and possibly G\textsubscript{i}, phospholipase C-mediated hydrolysis of polyphosphoinositides (10, 15, 17). The links between this or indeed other signalling pathways activated by oxytocin (14) and myometrial contractility are ill defined: they may involve regulation of both the intracellular free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) and the Ca\textsuperscript{2+} sensitivity of the contractile machinery.

In common with other smooth muscles (23), myometrial contractions are associated with increases in [Ca\textsuperscript{2+}]\textsubscript{i} (30). Ca\textsuperscript{2+} bound to calmodulin activates myosin light chain kinase, which then phosphorylates the regulatory myosin light chains, allowing them to rapidly bind to and detach from actin filaments and so generate tension. Although an increase in [Ca\textsuperscript{2+}]\textsubscript{i} is a major control of smooth muscle contraction, hormones can also enhance contractile activity without directly increasing [Ca\textsuperscript{2+}]\textsubscript{i} (29). Inhibition of myosin phosphatase causing a decrease in the rate of dephosphorylation of myosin light chain is a likely means of such Ca\textsuperscript{2+} sensitization (22, 23, 28). In permeabilized rat myometrium (8), oxytocin causes Ca\textsuperscript{2+} sensitization, but results from intact human tissue loaded with aequorin are contradictory (24). The effects of oxytocin on spontaneously active myometrium have not yet been examined under conditions that allow its influence on the [Ca\textsuperscript{2+}]\textsubscript{i}-tension relationship to be defined.

In the present study, myometrium taken before or after the onset of labor was loaded with fura 2, and the effects of oxytocin on the [Ca\textsuperscript{2+}]\textsubscript{i}-tension relationship were examined by simultaneous measurement of tension and [Ca\textsuperscript{2+}]\textsubscript{i} during phasic contractions.

METHODS

Simultaneous measurements of isometric tension and [Ca\textsuperscript{2+}]\textsubscript{i}. Myometrial biopsies were obtained with informed written consent and local ethical committee approval (LREC 89/56) at term caesarean section (37–42 wk gestation) either before or after the onset of labor. Indications for caesarean section included noncerebral presentation, previous caesarean section, failure to progress in labor, or fetal distress. Women had no significant medical conditions, and labor was defined as progressive (>2 cm) cervical dilatation accompanied by regular uterine contractions.

Small strips of myometrium (2 × 2 × 15 mm) were dissected so that the longitudinal axis aligned with the direction of the muscle fibers. Strips were incubated for 15 h at 20°C in Krebs-Henseleit solution (KHS) containing 50 μM fura 2-acetoxymethyl ester (Molecular Probes, Leiden, The Netherlands) dissolved in anhydrous dimethyl sulfoxide (10%) and pluronic acid (0.5%) and then were washed in KHS (30 min). KHS, equilibrated with 5% CO\textsubscript{2}-95% O\textsubscript{2}, had the following composition (in mM): 118 NaCl, 4.7 KCl, 1.2 CaCl\textsubscript{2}, 1.2 MgSO\textsubscript{4}, 25 NaH\textsubscript{2}CO\textsubscript{3}, 1.2 K\textsubscript{2}PO\textsubscript{4}, and 11 glucose, pH 7.4. Strips were mounted (Fig. 1A) in a polymethylacrylate cuvette within a Perkin-Elmer LS50B spectrofluorimeter. One end of the muscle was attached by cotton to an isometric tension recording system comprising a Fort-10 transducer (World Precision Instruments, Aston, UK: bandwidth 0–10 g) and an EpiCompact amplifier and data acquisition system.
RESULTS

Oxytocin increases [Ca$^{2+}$] sensitivity of human myometrium taken before or after the onset of labor. Although myometrial strips are intrinsically fluorescent at the wavelengths used to record fura 2 fluorescence, our results demonstrate that it is possible to simultaneously measure tension and [Ca$^{2+}$]. First, the fluorescence ratio of strips that had not been loaded with fura 2 was unaffected by contractions (Fig. 1B). Second, only muscles in which contractions were accompanied by reciprocal changes in the fura 2 fluorescence recorded at the two excitation wavelengths (340 and 380 nm) were analyzed (Fig. 1C). Third, in paired comparisons of myometrial strips (24 from 3 patients), fura 2 loading had no effect on spontaneous contractile activity: 11 of the 12 fura 2-loaded strips and 9 of the 12 control strips were spontaneously contractile; the peak tension (113 ± 39% of control, P > 0.05), frequency of contractions (109 ± 9%, P > 0.05), and AUC (130 ± 44%, P > 0.05) were all similar under the two conditions. Removal of extracellular Ca$^{2+}$ or addition of nimodipine (100 nM) abolished both spontaneous contractions and the changes in fluorescence ratio (not shown), in keeping with previous reports (6).

Comparison of the changes in [Ca$^{2+}$] and tension recorded during spontaneous contractions (60 min) of muscle strips taken before or after the onset of labor (Fig. 2) revealed no significant differences, nor were the effects of oxytocin different between the two samples (Table 1). Therefore, in all subsequent experiments, biopsies taken from patients before and after the onset of labor were pooled.

To better resolve the temporal relationship between changes in [Ca$^{2+}$] and tension during spontaneous contractions, recordings were made at 5 Hz. The increase in [Ca$^{2+}$] preceded the increase in tension (Fig. 3) by 436 ± 89 ms (n = 11) at the onset of a contraction.
and by $13 \pm 3$ s ($n = 17$) at its peak. These substantial delays, reflecting the cascade of biochemical events separating an increase in $[\text{Ca}^{2+}]_i$ from contraction, are similar to those observed in other smooth muscles (23, 31).

Our results establish that spontaneous contractile activity is dependent on $\text{Ca}^{2+}$ entry through L-type $\text{Ca}^{2+}$ channels (19, 32), that tissue taken before or after the onset of labor behaves similarly (although loss of labor characteristics during fura 2 loading cannot be excluded), and that fura 2 can be used to measure $[\text{Ca}^{2+}]_i$ in myometrium without interfering with its contractile activity.

Effect of oxytocin on phasic contractions. Because the half-maximal effect of oxytocin on myometrial contractile activity occurs when its concentration is $\sim 10$ nM (24, 26), this concentration was used in all subsequent experiments. Oxytocin had no significant effect on basal $[\text{Ca}^{2+}]_i$ or tension (Fig. 4); in paired comparisons of measurements during the first (pretreatment) and second (±oxytocin) hour of phasic contractile activity, the basal $[\text{Ca}^{2+}]_i$ and tension responses recorded from muscles stimulated with oxytocin were $98 \pm 3$ and $96 \pm 3\%$ ($n = 11$, $P > 0.05$ for each) of control responses, respectively. Control samples ($n = 4$) were not treated with oxytocin during the second hour to account for time-dependent changes in activity. Oxytocin did, however, increase the magnitude of both the tension and $[\text{Ca}^{2+}]_i$, recorded during phasic contractions (Fig. 5). Oxytocin had no significant effect on the frequency of contractions ($7.7 \pm 1.7$ and $5.1 \pm 0.8$ h$^{-1}$ before and after oxytocin, respectively, $P > 0.05$, $n = 11$), consistent with previous studies of myometrium in vitro (24).

Because oxytocin more markedly increased tension than $[\text{Ca}^{2+}]_i$, the relationship between $[\text{Ca}^{2+}]_i$ and tension was examined in detail.

Figure 6 shows the instantaneous relationship between $[\text{Ca}^{2+}]_i$ and tension during phasic contractions. A: high-resolution recording ($5$ Hz) of a single spontaneous contraction of myometrium demonstrates that the increase in $[\text{Ca}^{2+}]_i$ precedes tension. B: rising phase of the contraction shown on an expanded scale. Results are typical of those from 11 patients.

Table 1. Effects of oxytocin on phasic contractions from myometrium taken before or after the onset of labor

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Before Onset of Labor</th>
<th>After Onset of Labor</th>
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<tbody>
<tr>
<td></td>
<td>$[\text{Ca}^{2+}]_i$, nM</td>
<td>Tension, g</td>
</tr>
<tr>
<td>Basal</td>
<td>$61 \pm 9$</td>
<td>$1.52 \pm 0.21$</td>
</tr>
<tr>
<td>Peak increase</td>
<td>$67 \pm 3$</td>
<td>$1.88 \pm 0.42$</td>
</tr>
<tr>
<td>AUC/contraction</td>
<td>$7.5 \pm 1.8$</td>
<td>$0.23 \pm 0.10$</td>
</tr>
<tr>
<td>Frequency, h$^{-1}$</td>
<td>$6.2 \pm 0.7$</td>
<td>$10.2 \pm 4.5$</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>$64 \pm 10$</td>
<td>$1.25 \pm 0.19$</td>
</tr>
<tr>
<td>Basal</td>
<td>$79 \pm 7$</td>
<td>$3.23 \pm 0.64$</td>
</tr>
<tr>
<td>Peak increase</td>
<td>$16.9 \pm 6.2$</td>
<td>$0.71 \pm 0.28$</td>
</tr>
<tr>
<td>AUC/contraction</td>
<td>$4.1 \pm 0.6$</td>
<td>$7.0 \pm 1.9$</td>
</tr>
<tr>
<td>Frequency, h$^{-1}$</td>
<td>$2.0 \pm 1.4$</td>
<td>$3.4 \pm 2.1$</td>
</tr>
</tbody>
</table>

Data represent means ± SE. $[\text{Ca}^{2+}]_i$, intracellular free $\text{Ca}^{2+}$ concentration; AUC, area under the curve. Strips from 7 patients before and 4 after the onset of labor were each stimulated with oxytocin during the second hour of activity. Variables were compared by Students’ unpaired t-test before and after labor. There were no significant differences between responses from biopsies taken before and after the onset of labor ($P > 0.05$).
accommodate the variability between tissues from different patients (24) and yet allow quantitative analysis of the effects of oxytocin on the \([\text{Ca}^{2+}]_i\)-tension relationship, we adopted the following analysis. The average half-maximal increase in \([\text{Ca}^{2+}]_i\) for all muscle strips was 39 nM, and the tension recorded at this \([\text{Ca}^{2+}]_i\) was therefore compared on the rising and falling phases of the \([\text{Ca}^{2+}]_i\)-tension relationship. Comparison of these values from the last contraction before oxytocin addition with the first after its addition demonstrates that oxytocin significantly increases the tension only during the falling phase of the response (Table 2).

A simple means of examining changes in the \([\text{Ca}^{2+}]_i\)-tension relationship is provided by quantifying the maximal S of the rising and falling phases of the response (see METHODS). To eliminate problems resulting from slow changes in muscle properties during our protracted recordings, \([\text{Ca}^{2+}]_i\)-tension relationships were plotted for each contraction, S was calculated for each contraction during 2 h of activity, and S values were then expressed as percentages of the preceding contraction (Fig. 7). This form of analysis was applied to control myometrial strips and those treated with oxytocin (10 nM) for the second hour of phasic activity. The results demonstrate that there is no change in the S of the \([\text{Ca}^{2+}]_i\)-tension relationship between sequential control contractions but that, after oxytocin addition, the S abruptly increases and is thereafter maintained throughout the period of stimulation with oxytocin (Fig. 7).

Oxytocin increases the \([\text{Ca}^{2+}]_i\)-sensitivity of the contractile machinery. The results are so far consistent with oxytocin selectively increasing the \([\text{Ca}^{2+}]_i\)-sensitivity of the contractile apparatus during the falling phase of the contractile response. However, oxytocin also significantly increased the duration of the increase in \([\text{Ca}^{2+}]_i\) (and thereby the AUC), and this effect may have contributed to the change in the \([\text{Ca}^{2+}]_i\)-tension relationship (Figs. 5 and 6C and Table 1). To resolve the issue, \([\text{Ca}^{2+}]_i\) transients of similar duration were produced in the presence and absence of oxytocin by rapidly chelating extracellular \([\text{Ca}^{2+}]_i\) using EGTA during the rising phase of a phasic contraction (Fig. 8). Under these conditions, the \([\text{Ca}^{2+}]_i\) AUC was similar for control and oxytocin-treated muscle (107 ± 18% of control, \(n = 5\), \(P < 0.05\)), and the rate at which \([\text{Ca}^{2+}]_i\) monoexponentially returned to baseline was indistinguishable (time constant 18 ± 4 and 19 ± 4 s for control and oxytocin treated, respectively).
oxytocin still caused an increase in the tension recorded during a phasic contraction. In the presence of oxytocin, the peak and AUC tension were increased to 159 ± 13% (n = 5, P < 0.01) and 177 ± 31% (n = 5, P > 0.05) of their control values, and from the [Ca\(^{2+}\)]-tension relationship, oxytocin increased S to 203 ± 15% (n = 5, P < 0.05) of its pretreatment value (Fig. 8). These results establish that oxytocin increases the Ca\(^{2+}\) sensitivity of the contractile apparatus during phasic contractions independent of its ability to enhance the increase in [Ca\(^{2+}\)].

**DISCUSSION**

In the only previous simultaneous measurements of [Ca\(^{2+}\)] and tension in human myometrium, the luminescent indicator aequorin was used (24). This indicator has several limitations: the membrane must be transiently permeabilized to allow loading, it is difficult to calibrate, and it is not amenable to the protracted recording required for multiple measurements from a single strip. Fura 2, which has been used in rat myometrium (18–20), overcomes these limitations, and we have established that it can be used to quantify [Ca\(^{2+}\)] in spontaneously active human myometrium without interfering with its contractile behavior (Fig. 1).

Although labor is accompanied by profound changes in the physiology of the myometrium (3, 4, 9), our results suggest that the Ca\(^{2+}\) sensitivity of the contractile apparatus does not change in fura 2-loaded strips (Table 1), consistent with results from skinned myometrium of the rat (7). We cannot, however, entirely eliminate the possibility that some differences between labor and nonlabor issues have been lost during the 15 h taken to load strips with fura 2.

**Table 2. Oxytocin improves the [Ca\(^{2+}\)]-tension relationship only during the falling phase of contractions**

<table>
<thead>
<tr>
<th></th>
<th>Rising Phase</th>
<th>Falling Phase</th>
</tr>
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<tbody>
<tr>
<td>Pretreatment</td>
<td>0.81 ± 0.2</td>
<td>1.36 ± 0.33</td>
</tr>
<tr>
<td>Oxytocin (10 nM)</td>
<td>0.81 ± 0.3</td>
<td>2.25 ± 0.51*</td>
</tr>
</tbody>
</table>

Results are means ± SE of 8 independent determinations. Units are g. [Ca\(^{2+}\)]-tension plots similar to those shown in Fig. 6, B and D, were used to establish the tension recorded when [Ca\(^{2+}\)] had increased by 39 nM during the rising and falling phases of the [Ca\(^{2+}\)]-tension relationship in control and oxytocin-treated strips. Comparison is between the last phasic contraction recorded before oxytocin addition and the first recorded afterward. In parallel experiments, there were no differences between sequential control contractions (n = 4 patients, not shown). *Significant difference from control (P = 0.001).
Very high concentrations of oxytocin (µM) directly stimulate increases in [Ca^{2+}] and thereby tonic contractions (13). In our experiments, the characteristic phasic activity of normal myometrium (19, 30) was maintained by using a lower concentration (10 nM) of oxytocin, and the results pertain specifically to this concentration. Under these more physiological conditions, our results reveal two distinct modulatory effects of oxytocin on phasic activity.

First, oxytocin modestly increased the amplitude of the Ca^{2+} signal recorded during each spontaneous contraction (Table 1). We have not further addressed the mechanisms underlying this potentiation of the Ca^{2+} signals evoked by Ca^{2+} entry through L-type Ca^{2+} channels. The effect is not a consequence of oxytocin inhibiting Ca^{2+} removal from the cytosol, because after rapid removal of extracellular Ca^{2+}, rates of [Ca^{2+}] recovery were unaffected by oxytocin (Fig. 8). In addition, it cannot simply reflect release of Ca^{2+} stores by inositol 1,4,5-trisphosphate (IP_{3}) because, even during several hours of exposure to oxytocin (Fig. 4), its effects were manifest only during spontaneous contractions; neither basal tension nor [Ca^{2+}] was affected. IP_{3} receptors are stimulated by the concerted actions of IP_{3} and Ca^{2+} (25), and oxytocin may therefore have caused the formation of a subthreshold level of IP_{3} such that the increase in [Ca^{2+}] after spontaneous opening of L-type Ca^{2+} channels would synergize with it to cause release of intracellular Ca^{2+} stores and so amplify the Ca^{2+} signal. Alternatively, oxytocin, possibly via protein kinase C (16), may have increased the sensitivity of the L-type Ca^{2+} channels (11).

The second, and more striking, effect of this concentration of oxytocin was to selectively increase the tension evoked at each [Ca^{2+}] during the falling phase of each phasic contraction (Table 2). The only previous simultaneous measurements of [Ca^{2+}] and tension in pregnant human myometrium concluded that oxytocin had no effect on the [Ca^{2+}]-tension relationship at either the peak of the response or during tonic contractions (24). In our experiments, the effect of oxytocin on the [Ca^{2+}]-tension relationship of spontaneously active myometrium was independent of its ability to prolong the Ca^{2+} signal (Fig. 8). How might oxytocin, without directly increasing [Ca^{2+}], selectively increase the Ca^{2+} sensitivity of the contractile apparatus during only the later stages of each phasic contraction?

Oxytocin stimulates the mitogen-activated protein kinase (MAPK) cascade (14), and in some smooth muscles MAPK has been shown to phosphorylate caldesmon (1), thereby increasing the [Ca^{2+}]-sensitivity of the contractile apparatus (21). Such a mechanism would not, however, readily explain why oxytocin increases the [Ca^{2+}]-sensitivity during only the falling phase of the response. A more likely mechanism would involve inhibition of myosin light chain phosphatase activity (23), the effect of which would be more pronounced after substantial phosphorylation of myosin light chains. Rho and its associated kinase (28), arachidonic acid, G proteins, and protein kinase C (23) have each been implicated in linking receptors to inhibition of myosin light chain phosphorylation, but the links with the oxytocin receptor remain to be defined.

We conclude that oxytocin selectively increases the [Ca^{2+}]-sensitivity of the contractile apparatus during only the falling phase of a contraction, possibly by inhibition of myosin phosphatase. Selective manipulation of the mechanisms responsible for the [Ca^{2+}]-sensitization may ultimately provide additional means of controlling myometrial contractility for induction of labor or treatment of preterm labor.

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Fig. 8. Oxytocin increases the [Ca^{2+}] sensitivity independent upon its effect on [Ca^{2+}]. Contractions were recorded in the absence (A) or presence (C) of oxytocin, and extracellular Ca^{2+} was reduced rapidly during the subsequent contraction to ensure that the [Ca^{2+}] signals were similar irrespective of the presence of oxytocin. Resulting [Ca^{2+}]-tension plots (B and D) demonstrate that oxytocin retains its ability to increase the [Ca^{2+}]-sensitivity of the falling phase of the response independent of its ability to prolong the [Ca^{2+}] signal. •, [Ca^{2+}]-tension relationship in Krebs-Henseleit solution; ○, responses when extracellular Ca^{2+} was rapidly removed during contraction. Results are typical of 5 similar experiments. Some symbols have been omitted from the [Ca^{2+}]-tension plots (B and D) for clarity.

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