Br. J. Pharmac. (1985), **84**, 175–183

The response of non-pregnant rat myometrium to oxytocin in Ca-free solution

F. Ashoori, A. Takai & T. Tomita

Department of Physiology, School of Medicine, Nagoya University, Nagoya 466, Japan

1 The contractile response of the longitudinal muscle of non-pregnant rat myometrium to oxytocin (0.2–20 nM) consisted of a phasic and a tonic component. Ca-removal abolished the phasic component but a tonic contraction could be evoked without reduction of amplitude for 50 h. Exceptionally, the tonic contraction also disappeared gradually in Ca-free medium containing 2 mM EGTA.

2 When oxytocin was repeatedly applied in the absence of Ca, the response became at first progressively larger before reaching a steady state. Transient addition of Ca to the medium reduced the size of the subsequent oxytocin contraction.

3 In Ca-free medium, the tissue lost Ca slowly, but it still contained 40 μmol kg^{-1} after 6 h and roughly 1 μmol kg^{-1} wet weight after 24 h exposure. ^{45}Ca efflux was marginally increased by oxytocin (20 nM).

4 Caffeine (5–30 mM) produced no contraction, but slightly reduced the resting tension and strongly inhibited the oxytocin response both in the presence and in the absence of Ca. Caffeine also blocked the contraction induced by Ca added to Ca-free 40 mM K solution. However, pretreatment with caffeine (30 mM) had no effect on the following oxytocin response.

5 A calmodulin antagonist, trifluoperazine (1–10 μM) suppressed strongly the Ca-induced contraction, but had only a weak effect on the oxytocin response in Ca-free medium. Chlorpromazine (10–100 μM) and fluphenazine (10–30 μM) had similar effects. A different type of antagonist, N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide (W-7) (0.1 mM) almost completely blocked responses to both oxytocin and to Ca, but recovery of the Ca-induced contraction was much better than that of the oxytocin response in Ca-free solution.

6 Since no evidence was found for intracellular Ca release by oxytocin, and as there were several differences between the effects of calmodulin antagonists on the oxytocin response and on the Ca-induced response of similar size, the possibility remains that some Ca-independent process is involved in the contractile response to oxytocin observed in Ca-free solution.

Introduction

The contraction of many smooth muscles depends on the presence of external Ca and disappears following Ca removal, although the rate of disappearance differs in different smooth muscle types. The speed of disappearance probably reflects the rate of depletion of an intracellular Ca store. However, it has recently been reported that the contraction of some smooth muscles is extremely resistant to Ca removal. For example, in the smooth muscle of the ear artery, main pulmonary artery and saphenous artery of the rabbit, noradrenaline and histamine can produce a contraction, consisting of a phasic and a small tonic component, in Ca-free solution containing 2 mM EGTA. When the agonist is applied repeatedly, the phasic component is obtained only on the first application, while the tonic component remains constant for more than 80 min (Casteels *et al.*, 1981). The same result has been obtained in the rat aorta with noradrenaline (Heaslip & Rahwan, 1982). Fluoride (1–12 mM) is also known to produce a slowly rising tonic contraction, which is resistant to Ca removal, in rabbit arteries (Casteels *et al.*, 1981) and in the bovine coronary artery (Fermum *et al.*, 1977).

Similarly, the oxytocin response in the rat myometrium (Sakai & Uchida, 1980; Sakai *et al.*, 1981; 1982) and the noradrenaline response in the rat vas deferens (Ashoori & Tomita, 1983) can be observed during prolonged exposure to Ca-free solution con-

taining 0.5–3 mM EGTA. The size of the contraction tends to increase with time in the absence of Ca, and it is suppressed by pretreatment with 2.4 mM Ca or during addition of a low concentration of Ca (0.03 mM for the myometrium and 0.2 mM for the vas deferens).

These contractions are generally interpreted as being due to Ca released from an intracellular store, which cannot be wholly depleted. However, since intracellular free Ca is considered not to increase sufficiently to elicit a contraction in the presence of fluoride, a special Ca-independent mechanism has been postulated (Casteels *et al.*, 1981).

The aim of the present experiments was to obtain evidence for the depletion of intracellular Ca stores and to compare the properties of the oxytocin response in Ca-free medium with the contraction induced by Ca in the longitudinal muscle of the non-pregnant rat myometrium.

Methods

Non-pregnant Wistar rats (200–250 g) were stunned and bled. After isolating the uterus, muscle strips, about 1 mm wide and 7 mm long, were obtained and the longitudinal muscle dissected out under a microscope. The hormonal state was checked by examining the vaginal smear.

The methods of recording tension and of superfusing were the same as those described previously (Ashoori & Tomita, 1983). The preparation was mounted in an organ bath (1 ml in volume) through which solution flowed at a constant rate of 2 ml min^{-1}. The tension was isometrically recorded using a strain gauge. The preparation was equilibrated in normal solution for 1 h and the resting tension was adjusted to 0.05 g before starting the experiments.

The normal solution had the following composition (mM): NaCl 136.9, $KHCO_3$ 5.9, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 11.8. In order to produce a contraction by the readdition of Ca, the following Ca-free solution containing excess K was used (mM): NaCl 106.3, KCl 34, $KHCO_3$ 5.9, $MgCl_2$ 1,2, glucose 11.8 and EGTA 2. When a low concentration of Ca (0.05–1 mM) was introduced, EGTA was removed 10 min before the addition of Ca. The experiments were carried out at 35°C.

The tissue Ca contents were measured by an atomic absorption spectrophotometer (Hitachi 208), after digesting the tissues with HNO_3 and $HClO_2$ at 100°C for 3–6 h. For ^{45}Ca experiments, the tissues (50–100 mg) were loaded with normal solution containing $1.5–5.1 \times 10^{-8}$ M ^{45}Ca for 22–24 h at room temperature (20–23°C). ^{45}Ca efflux was obtained by sequentially passing the preparations at 10–20 min intervals through a series of scintillation vials which contained 1 ml Ca-free, 40 mM K, 2 mM EGTA solution, kept at 35°C, and the activity was measured with the Aloka mini-scintillation counter. At the end of efflux measurements, the total ^{45}Ca remaining in the tissue was measured after digesting the tissues with 30–35% H_2O_2 at 100°C for 3–5 h.

Results

In the longitudinal muscle of the non-pregnant rat myometrium, oxytocin (0.2 nM) produced a contraction which usually consisted of phasic and tonic components. The pattern of response differed to some degree in different preparations, but there was no clear relationship between the response and sexual cycle or between the response and the location from which the preparation was isolated. In pregnant and ovariectomized rats, the oxytocin response was very poor.

When Ca was removed from the medium, external K concentration was increased to 40 mM and 2 mM EGTA was added. In this solution, the phasic contraction to oxytocin disappeared and the tonic contraction was reduced. In Figure 1, some examples of the oxytocin responses obtained before and after removal of external Ca are shown. After observing the control response, oxytocin was repeatedly applied for 4 min at 20 min intervals in Ca-free solution containing 2 mM EGTA. Usually, the external K concentration was increased to 40 mM when Ca was removed, but the oxytocin response was much the same in solutions containing normal (5.9 mM) and excess (40 mM) amounts of K. In most preparations, the oxytocin response developed slowly and its size and the rate of rise gradually increased with each application, as shown in Figure 1a, until, after 4–6 applications, a steady state was reached. A similar response, resistant to Ca removal, has been described for the oestrogen-treated ovariectomized rat (Sakai *et al.*, 1981; 1982). Occasionally, the oxytocin response was slowly but markedly reduced after removing Ca from the medium (Figure 1b and c). In preparations in which the oxytocin response became very small after Ca removal, the rate of tension development caused by oxytocin was relatively fast, the peak being reached within 2 min.

No apparent correlation was found between the sexual cycle and the response pattern in normal or in Ca-free solution, but preparations taken from the same animal showed a similar type of response. In the following experiments, only the responses which were resistant to Ca removal will be described.

Figure 2 shows the responses to different concentrations of oxytocin (0.2–20 nM) in normal Krebs solution and in Ca-free solution. Oxytocin was ap-

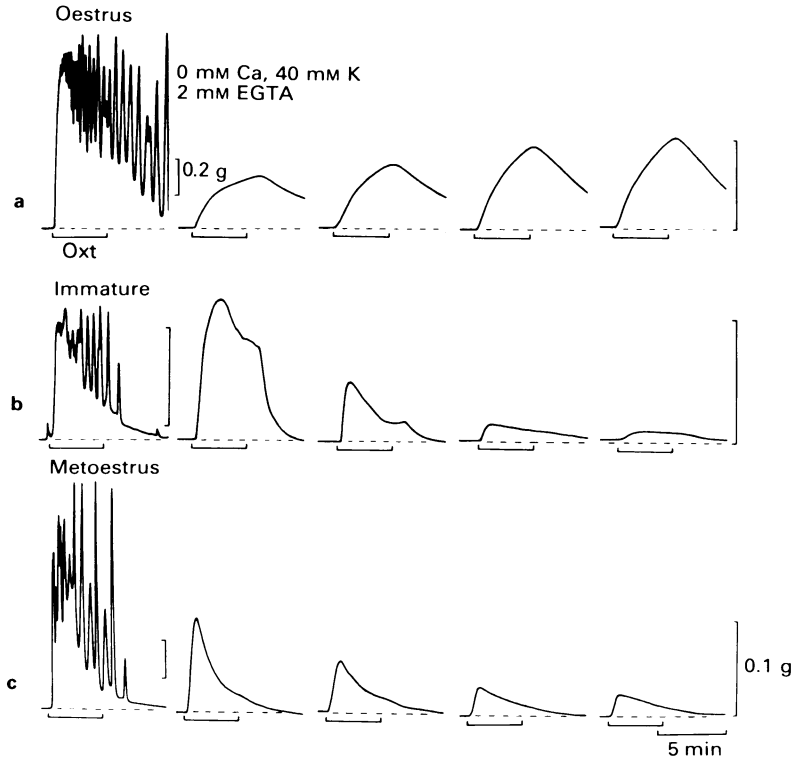

Figure 1 Examples of different mechanical responses of the longitudinal muscle of rat myometrium to oxytocin (Oxt; 10 nM), in the presence and in the absence of Ca. Oxytocin was applied for 4 min (horizontal bars) at intervals of 20 min. In most preparations, the response was increased with time of exposure to Ca-free solution as shown in (a), but in some preparations the oxytocin response declined. Note different calibrations for the responses in normal and Ca-free solution.

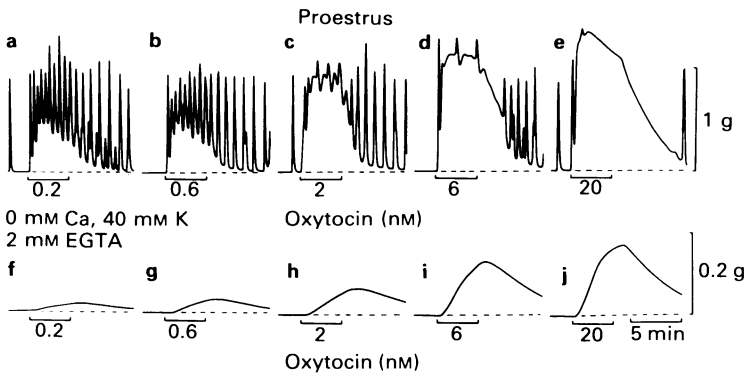

Figure 2 Mechanical response to oxytocin at different concentrations in the presence and absence of Ca. Oxytocin (0.2–20 nM) was applied for 4 min at 20 min intervals, before (a–e) and after Ca removal (f–j) in the same preparation.

plied for 4 min at 20 min intervals by successively increasing its concentration. After observing the response to 20 nM oxytocin in normal medium (Figure 2e), Ca was removed, and 20 min later the oxytocin applications were repeated but recorded with a 4 times higher gain. The threshold concentration was apparently not different but, due to the presence of phasic activity in normal solution and also due to the time-dependent increase of the response in Ca-free solution, concentration-response curves in the presence and absence of Ca were difficult to compare. The maximum tension produced in Ca-free solution was 10–30% of the tonic component of the oxytocin response in normal Krebs solution.

If the response in Ca-free solution is assumed to be a result of Ca release from an intracellular store, one would expect some reduction of the response with a prolonged exposure to Ca-free solution because of depletion of the store. Therefore, in four preparations, the oxytocin response was observed from time to time during continuous superfusion with Ca-free solution for more than 46 h. The results of such an experiment are shown in Figure 3, and the peak tension produced by a 4 min application of 20 nM oxytocin obtained from two preparations is plotted in Figure 4. At first, following Ca removal, the response became successively larger with repetitive oxytocin administration at 20 min intervals, as already mentioned, but when oxytocin was repeatedly applied 6 h after Ca removal, following a long interval of more than 3 h since the preceding oxytocin treatment, the response became successively smaller with repetitive administration of oxytocin, and this tendency continued. Nevertheless, there was no clear indication of a decrease in the response in Ca-free solution for at least 50 h.

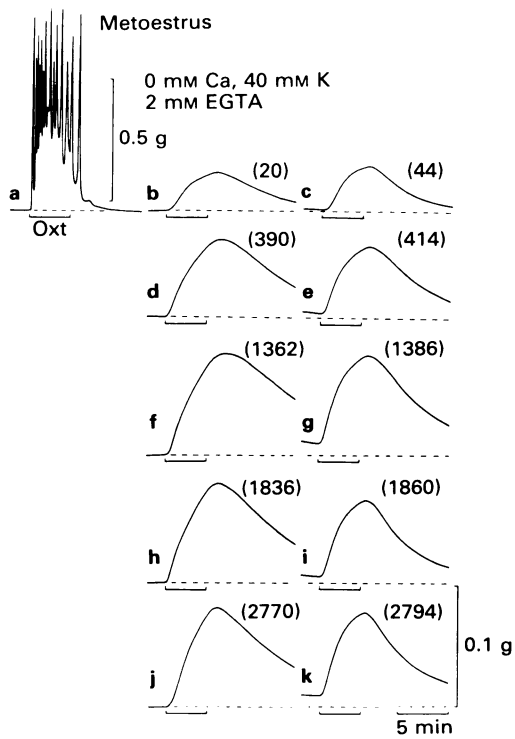

Figure 3 Responses to oxytocin (Oxt; 20 nM) during prolonged exposure to Ca-free solution. After observing the oxytocin response in normal solution (a), continuous superfusion with Ca-free solution was started. Time (in min) of oxytocin application (horizontal bars) after Ca removal is indicated by the figures in parentheses.

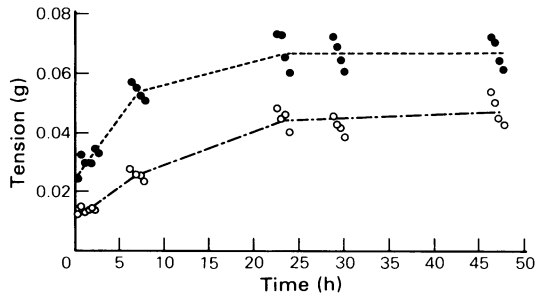

Figure 4 Peak tension of oxytocin (20 nM) responses plotted against the time of exposure to Ca-free solution, based on similar experiments to those shown in Figure 3. Responses in two different preparations are shown (filled circles: metoestrous; open circles: oestrus).

It is possible that the tissue can retain intracellular Ca during repeated contractions and that Ca is recycled between the cytosol and the store without a significant loss. Thus, changes in tissue Ca content in Ca-free solution were measured with an atomic absorption spectroscope. The results are shown in Table 1. The decrease between 2 and 6 h was roughly exponential, the average half-time being 154 min ($n = 3$). The Ca content fell below a detectable concentration (to approximately 1 μmol kg^{-1} wet weight using more than 100 mg) after 24 h exposure to Ca-free solution. All isotope experiments showed similar results, although the variation in flux rate was large in

Table 1 Ca content of rat myometrium in Ca-free solution measured using atomic absorption spectrophotometry

Time in Ca-free medium (min)	*Ca content* (mmol kg^{-1} wet weight; $n = 3$)
30	0.31 ± 0.01
120	0.12 ± 0.02
240	0.07 ± 0.03
360	0.04 ± 0.01

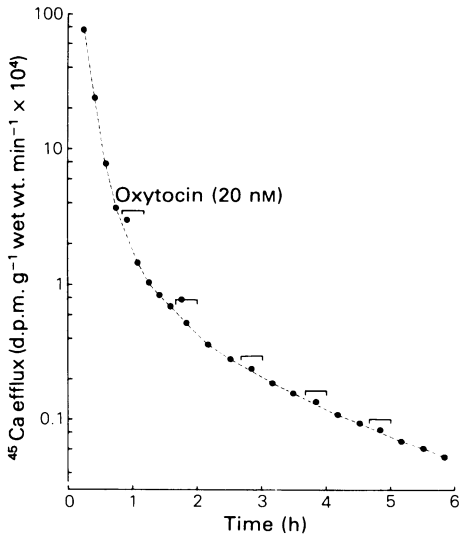

Figure 5 Effects of oxytocin (20 nM) on ^{45}Ca efflux. ^{45}Ca was loaded in normal solution containing 3×10^{-8} M ^{45}Ca at room temperature for 22 h, and the efflux was measured in Ca-free solution at 35°C. Oxytocin application indicated by horizontal bars.

different preparations. The half time obtained from the efflux curve between 4 and 6 h was 178 ± 24 min (the rate constant: 0.24 ± 0.04 h^{-1}, $n = 5$). Thus, it was concluded that the preparation is clearly loosing Ca, and that there is no close relationship between the tissue Ca content and the mechanical response to oxytocin in Ca-free solution, although the preparations may still contain enough Ca for a small contraction even after a 24 h incubation in Ca-free solution.

Oxytocin (20 nM) had an insignificant effect on ^{45}Ca efflux, as shown in Figure 5. There was a tendency for ^{45}Ca efflux to increase very slightly during oxytocin administration, but it was difficult to decide whether this was a result of intracellular Ca release responsible for the contraction, or a mechanical artefact due to squeezing ^{45}Ca from the extracellular space by the contraction.

Figure 6 shows effects of the re-inclusion of Ca in the medium. After observing the oxytocin (20 nM) response in the presence of Ca (a) and also in the absence of Ca (b and c), 0.1 mM Ca was included in the medium for 4 min. This low Ca concentration produces a small slowly developing contraction (d). The response (e) to oxytocin (20 nM) observed 20 min after the transient administration of Ca was much smaller than that produced before the application of Ca. The second response (f) to Ca was larger than the first. The Ca response was always increased when induced repeatedly at 20 min intervals in Ca-free, excess K solution, even without interposing an oxytocin response. In the presence of oxytocin (g), the response to Ca was markedly potentiated, but the response (h) to oxytocin produced 20 min after Ca treatment was much smaller than those (c and i) produced without preceding Ca treatment. Thus, Ca pretreatment had an antagonistic effect on the oxytocin response induced in Ca-free medium, confirming previous findings (Sakai & Uchida, 1980).

In vascular smooth muscle, caffeine is known to release Ca from the intracellular store which is involved in the mechanical response to noradrenaline

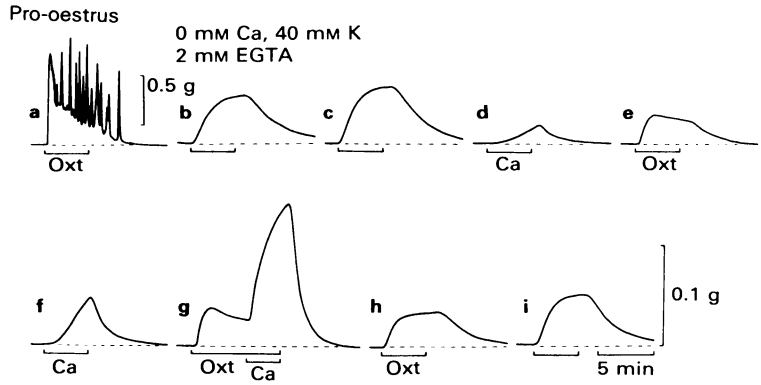

Figure 6 Effects of the transient re-incorporation of Ca (0.1 mM) in the bathing solution on the oxytocin response. (a) Response to oxytocin (20 nM) in the presence of Ca (2.4 mM); (b and c) in the absence of Ca. (d) Response to the addition of 0.1 mM Ca in the medium for 4 min. (e) Oxytocin response in Ca-free solution, 20 min after Ca had been removed from the medium. (f) The 2nd response to Ca (0.1 mM), (g) the effect of Ca, added to the medium in the presence of oxytocin. (h and i) Responses to successive applications of oxytocin in Ca-free solution at 20 min intervals. 10 min before each Ca application, EGTA was removed, otherwise Ca-free solution contained 2 mM EGTA.

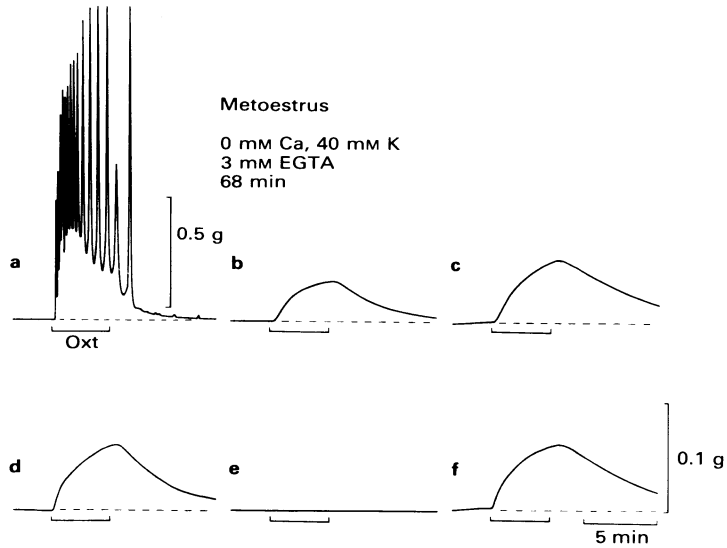

Figure 7 Effects of caffeine (30 mM) on the response to oxytocin (Oxt; 20 nM) in Ca-free solution. (a) Oxytocin response in normal solution. Caffeine was applied for 20 min between the first (b) and the second application (c) of oxytocin in Ca-free solution. (c and d) Oxytocin responses 10 min and 34 min after caffeine treatment. (e) Inhibition of the oxytocin response by caffeine (30 mM) and (f) the recovery of the response 10 min after washing out caffeine.

(Deth & Casteels, 1977; Deth & Lynch, 1981; Itoh *et al.*, 1981; 1982; 1983). In these preparations, the noradrenaline response observed in Ca-free solution is inhibited by pretreatment with caffeine because the Ca store is depleted. In the rat myometrium, however, there was no such inhibitory effect of caffeine treatment (up to 30 mM) on the oxytocin response in Ca-free solution. In Figure 7, after observing the

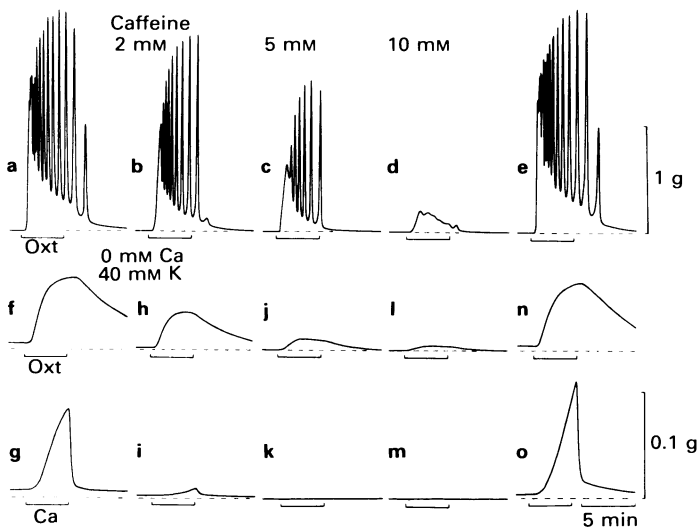

Figure 8 Effects of different concentrations of caffeine on the responses to oxytocin (Oxt; 20 nM) in the presence (b, c and d) and absence (h, j and l) of Ca, and on the Ca-induced contraction (i, k and m). In (g–o) Ca (0.1 mM) was added to Ca-free, 40 mM K solution, from which EGTA had been removed 10 min before the addition of Ca. Experimental sequence was in alphabetical order. Since the re-inclusion of Ca in the medium has an inhibitory effect on a subsequent oxytocin response in Ca-free solution, Ca and oxytocin responses were repeated at least twice before the drugs were applied to stabilize the responses in this and the following figures.

oxytocin response in normal solution (a) and in Ca-free solution (b), 30 mM caffeine was applied for 20 min. Caffeine produced no contracture, but only a small decrease in resting tension. The response to oxytocin (c) applied 10 min after washing out caffeine was larger than the first response, as usually found without caffeine treatment. However, the oxytocin response was completely abolished in the presence of 30 mM caffeine (e), but the recovery was perfect (f).

Caffeine (2–10 mM) suppressed reversibly not only the oxytocin responses in the absence of Ca (Figure 8h, j and l), but also those in the presence of Ca (Figure 8b, c and d). Furthermore, the contraction induced by the addition Ca (0.1 mM) to medium containing excess K (40 mM) was also strongly inhibited by caffeine (Figure 8i, k and m). Thus, although caffeine has a strong suppressive effect on the contraction itself, there is no evidence that this results from a depletion of an intracellular Ca store.

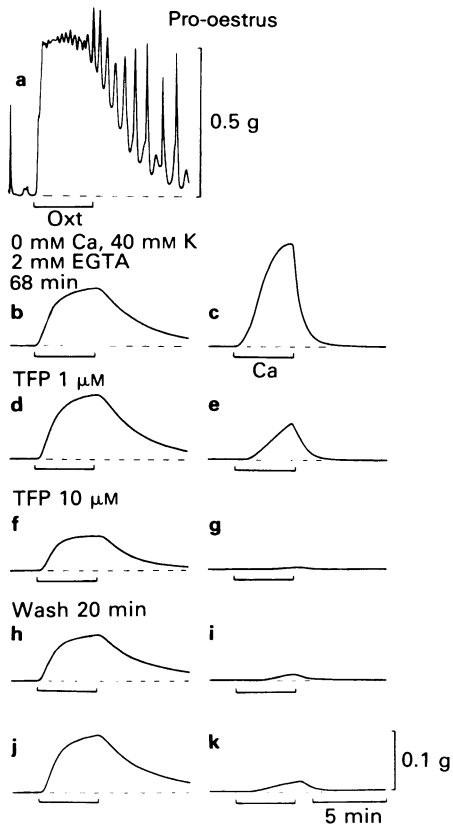

Figure 9 Effects of trifluoperazine (TFP) on the responses to oxytocin (Oxt; 20 nM) in Ca-free solution and on the contraction induced by the addition of Ca (0.1 mM) in the medium. Left column (a,b,d,f,h and j), oxytocin responses; right column (c,e,g,i and k), Ca responses. Note strong suppression and poor recovery of the Ca-induced contraction by TFP compared with the oxytocin response.

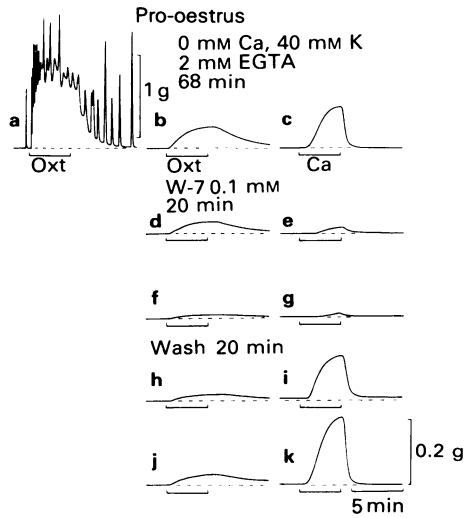

Figure 10 Effects of W–7 on the responses to oxytocin (Oxy; 20 nM) (b,d,f,h and j) in Ca-free solution and on the contraction induced by the addition of Ca (0.1 mM) in the medium (c,e,g,i and k). The same experimental procedure as in Figure 9, but 0.1 mM W–7 was applied for a longer period (d–g). Note similar suppression of both responses by W–7, but a better recovery of the Ca-induced response.

According to the prevailing hypothesis, the contraction of smooth muscle is caused by phosphorylation of myosin mediated by myosin light chain kinase, and this process is activated by a reaction between Ca and calmodulin contained in the myosin light chain kinase (Hartshorne & Siemankowski, 1981). Thus, it would be expected that calmodulin antagonists suppress the contraction. Phenothiazine derivatives, such as trifluoperazine (TFP) are known to antagonize calmodulin. Figure 9 shows effects of TFP on the oxytocin response in Ca-free solution and on the contraction induced by the addition of Ca (1 mM). After observing the control response (a) to oxytocin in the presence of Ca, the preparation was exposed to Ca-free, 40 mM K medium. In this solution, oxytocin and Ca were applied alternately at 20 min intervals. TFP (1 μM) was applied 10 min after the second application of Ca (c), and 10 min later the responses to oxytocin and Ca were simultaneously repeated in the presence of 1 μM TFP (d,e) and 10 μM TFP (f,g). The Ca-induced contraction was strongly inhibited while the oxytocin response was only moderately reduced by TFP (10 μM). A clear recovery of the oxytocin response was observed after removal of TFP, but recovery of the Ca-induced response was very poor.

The effects of other phenothiazine derivatives, fluphenazine (10–30 μM) and chlorpromazine (50–100 μM) were essentially the same as those of TFP. However, another calmodulin antagonist, N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide (W-7), which is considered to be more specific (Asano *et al.*, 1982), had different effects from those of the phenothiazine derivatives as shown in Figure 10. W-7 (0.1 mM) slowly suppressed both the oxytocin response (d,f) and the Ca-induced response (e,g) in parallel, but the recovery of the Ca-induced responses (i,k) was much better than that of the oxytocin response (h,j). Thus, there was a clear difference between the suppressive effects of calmodulin antagonists on the oxytocin response in Ca-free solution and on the contraction induced by Ca, even though the magnitude of both tension responses was similar before treatment with the antagonists.

Discussion

In vascular smooth muscles, noradrenaline is thought to release Ca from an intracellular store. There are several lines of evidence for this idea. A transient contraction can be produced by noradrenaline in Ca-free medium, but its magnitude is gradually reduced with an increased time of exposure to Ca-free medium, suggesting a slow depletion of Ca from the store (Deth & Van Breemen, 1977; Droogmans *et al.*, 1977). When the contraction has once been produced by noradrenaline, the second response to noradrenaline becomes very small or negligible, probably due to a limited amount of stored Ca (Deth & Casteels, 1977; Deth & Van Breemen, 1977; Van Breemen & Siegel, 1980). The intracellular Ca store is considered to be sarcoplasmic reticulum rather than mitochondria, based on electron probe analysis (Somlyo *et al.*, 1982).

The contraction induced by oxytocin in the myometrium is extremely resistant to Ca removal. The contraction is actually increased with time after Ca removal and it is suppressed by re-addition of Ca. Furthermore, the tissue clearly loses Ca in Ca-free medium under the same conditions in which the oxytocin response is increasing or remains more or less constant. Thus, if the oxytocin response is due to intracellular release of Ca, the property of the Ca store in the myometrium differs significantly from that in typical vascular smooth muscle. On the other hand, it may be possible that the contraction is caused by a mechanism in which Ca is not involved, as proposed by Casteels *et al.* (1981).

Caffeine (5 mM) causes the release of intracellular Ca, causing a transient contraction in vascular smooth muscle exposed to Ca-free medium (Deth & Casteels, 1977; Deth & Lynch, 1981; Itoh *et al.*, 1981; 1983). The Ca store seems to be the same for caffeine and noradrenaline responses, since noradrenaline cannot produce a contraction in Ca-free solution after depletion of the Ca store by pretreatment with caffeine. On the other hand, in the myometrium of pregnant mouse, caffeine is known to have an antagonistic effect on the contraction (Osa, 1973), and thus, it is not clear whether caffeine also releases Ca intracellularly in this preparation. A similar inhibitory effect of caffeine was observed in the non-pregnant rat myometrium in the present experiments. It is very likely that the inhibition of phosphodiesterase by caffeine increases intracellular cyclic AMP and that this may result in direct suppression of contraction (Conti & Adelstein, 1981; Rüegg *et al.*, 1981; Rüegg & Paul, 1982). In accord with this idea, other xanthine derivatives and dibutyryl cyclic AMP also inhibit the oxytocin response of myometrium (Sakai *et al.*, 1981) and the noradrenaline response of vas deferens in Ca-free solution (Ashoori & Tomita, 1983). The inhibitory effect of caffeine mediated by cyclic AMP may be much stronger than the contractile effect due to Ca release in the myometrium. However, the fact that the oxytocin response remains the same, or becomes even larger, after treatment with caffeine suggests either that caffeine does not release Ca or that the released Ca is effectively taken up again. Another possibility would be that the oxytocin response may be mediated by a Ca-independent process which is inhibited by cyclic AMP.

If the oxytocin response observed in Ca-free solution is caused by an increase in the intracellular free Ca concentration, one would expect it to be suppressed by calmodulin antagonists in the same way as the contraction induced by application of Ca. Phenothiazine derivatives, trifluoperazine, fluphenazine and chlorpromazine all inhibit the Ca-induced contraction much more strongly than the oxytocin response. This may suggest that the mechanism underlying the oxytocin response differs from that for the Ca-induced contraction. However, since chlorpromazine is supposed to have a blocking action on the Ca channel at the plasma membrane (Karaki *et al.*, 1982), one cannot draw a firm conclusion concerning this problem. Another type of calmodulin antagonist, W-7 suppresses both the oxytocin response and the Ca-induced contraction to a similar degree, suggesting that Ca may be responsible for the oxytocin response. However, since the recovery is poor for the oxytocin response, W-7 probably affects some other process in addition to a calmodulin-mediated process involved in the Ca-induced contraction.

Recently, we have found that a vasodilator substance, N-(2-guanidinoethyl)-5-isoquinolinesulphonamide, selectively inhibits the oxytocin re-

sponse in the absence of Ca, while this substance has very weak effects on the oxytocin response in the presence of Ca and on the Ca-induced contraction (unpublished observation). Thus, there are several differences between the oxytocin response in Ca-free medium and the Ca-induced contraction with respect to their sensitivity to drugs. Although these pharmacological analyses must be interpreted with reservation, the oxytocin response seems somewhat difficult to explain by the generally accepted mechanism in which Ca plays an essential role.

We are grateful to Professor Edith Bülbring, Oxford, for improving the manuscript. This work was partly supported by the Grant in Aid for Scientific Research from the Ministry of Education, Japan (Project numbers 56370006 and 58370007).

References

ASANO, M., SUZUKI, Y. & HIDAKA, H. (1982). Effects of various calmodulin antagonists on contraction of rabbit aortic strips. *J. Pharmac. exp. Ther.*, **220,** 191–196.

ASHOORI, F. & TOMITA, T. (1983). Mechanical response to noradrenaline in calcium-free solution in the rat vas deferens. *J. Physiol.*, **338,** 165–178.

CASTEELS, R., RAEYMAEKERS, L., SUZUKI, H. & VAN ELDERE, J. (1981). Tension response and Ca-45 release in vascular smooth muscle incubated in Ca-free solution. *Pflügers Arch.*, **391,** 139–145.

CONTI, M.A. & ADELSTEIN, R.S. (1981). The relationship between calmodulin binding and phosphorylation of smooth muscle kinase by the catalytic subunit of 3′:5′-cAMP-dependent protein kinase. *J. biol. Chem.*, **256,** 3178–3181.

DETH, R. & CASTEELS, R. (1977). A study of releasable Ca fractions in smooth muscle cells of the rabbit aorta. *J. gen. Physiol.*, **69,** 401–416.

DETH, R.C. & LYNCH, C.J. (1981). Mobilization of a common source of smooth muscle Ca^{2+} by norepinephrine and methylxanthines. *Am. J. Physiol.*, **240,** C239-C247.

DETH, R. & VAN BREEMEN, C. (1977). Agonist induced release of intracellular Ca^{2+} in the rabbit aorta. *J. memb. Biol.*, **30,** 363–380.

DROOGMANS, G., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *J. gen. Physiol.*, **70,** 129–148.

FERMUM, R., MEISEL, P. & KLINNER, U. (1977). Versuche zum Wirkungsmechanismus von Gefässspasmolytika. 3. Wirkung von Nitroprussid-Natrium, Nitroglyzerin, Prenylamin und Verapamil an der Fluorid-induzierten Kontraktur isolierter Koronararterien. *Acta biol. med. Germ.*, **36,** 245- 255.

HARTSHORNE, D.J. & SIEMANKOWSKI, R.F. (1981). Regulation of smooth muscle contraction. *Ann. Rev. Physiol.*, **43,** 519–530.

HEASLIP, R.J. & RAHWAN, R.G. (1982). Evidence for the existence of two distinct pools of intracellular calcium in the rat aorta accessible to mobilization by norepinephrine. *J. Pharmac. exp. Ther.*, **221,** 7–13.

ITOH, T., KAJIWARA, M., KITAMURA, K. & KURIYAMA, H. (1982). Roles of stored calcium on the mechanical response evoked in smooth muscle cells of the porcine coronary artery. *J. Physiol.*, **322,** 107–125.

ITOH, T., KURIYAMA, H. & SUZUKI, H. (1981). Excitation-contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. *J. Physiol.*, **321,** 513–535.

ITOH, T., KURIYAMA, H. & SUZUKI, H. (1983). Differences and similarities in the noradrenaline- and caffeine-induced mechanical responses in the rabbit mesenteric artery. *J. Physiol.*, **337,** 609–629.

KARAKI, H., MURAKAMI, K., NAKAGAWA, H., OZAKI, H. & URAKAWA, N. (1982). Effects of calmodulin antagonists on tension and cellular calcium content in depolarized vascular and intestinal smooth muscles. *Br. J. Pharmac.*, **77,** 661–666.

OSA, T. (1973). The inhibitory action of caffeine on the smooth muscles of mouse myometrium and guinea-pig ileum. *Jap. J. Physiol.*, **23,** 199–216.

RÜEGG, J.C. & PAUL, R. (1982). Vascular smooth muscle. Calmodulin and cyclic AMP-dependent protein kinase alter calcium sensitivity in porcine carotid skinned fibers. *Circulation Res.*, **50,** 394–399.

RÜEGG, J.C., SPARROW, M.P. & MRWA, U. (1981). Cyclic-AMP mediated relaxation of chemically skinned fibers of smooth muscle. *Pflügers Arch.*, **390,** 198–201.

SAKAI, K., HIGUCHI, K., YAMAGUCHI, T. & UCHIDA, M. (1982). Oxytocin-induced Ca-free contraction of rat uterine smooth muscle: effects of preincubation with EGTA and drugs. *Gen. Pharmac.*, **13,** 393–400.

SAKAI, K. & UCHIDA, M. (1980). A calcium reversal phenomenon: differentiation of excitatory and inhibitory roles of Ca in uterine smooth muscle contraction. *Jap. J. Pharmac.*, **30,** 394–396.

SAKAI, K., YAMAGUCHI, T. & UCHIDA, M. (1981). Oxytocin-induced Ca-free contraction of rat uterine smooth muscle: effects of divalent cations and drugs. *Arch. int. Pharmacodyn.*, **250,** 40–54.

SOMLYO, A.P., SOMLYO, A.V., SHUMAN, H. & ENDO., M. (1982). Calcium and monovalent ions in smooth muscle. *Fedn. Proc.*, **41,** 2883–2890.

VAN BREEMEN, C. & SIEGEL, B. (1980). The mechanism of β-adrenergic activation of the dog coronary artery. *Circulation Res.*, **46,** 426–429.

(Received June 18, 1984.
Revised September 4, 1984.)