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Contractions of rat uterine smooth muscle induced by acetylcholine and angiotensin II in Ca^{2+}-free medium

C. Lalanne, C. Mironneau, J. Mironneau & J.P. Savineau

Laboratoire de Physiologie Cellulaire, Institut de Biochimie Cellulaire et Neurochimie du C.N.R.S. et Université de Bordeaux 2, 1 rue Camille Saint-Saëns, 33077 Bordeaux, France

1 The effects of acetylcholine (ACh, 10^{-4}M) and angiotensin II (Ang II, 10^{-6} M) have been studied on the mechanical and electrical activities of rat myometrial strips perfused in Ca^{2+}-free EGTA-containing solutions.

2 Both ACh and Ang II produced transient contractions, the amplitude of which can be taken as a measurement of the amount of Ca^{2+} present in a drug-sensitive Ca^{2+} store. The degree of filling of this store depended on the external Ca^{2+} concentration, and on the presence of contractile responses during the Ca^{2+} loading period. The existence of two pathways (either direct or transcytoplasmic) is suggested for Ca^{2+} uptake into the internal Ca^{2+} store.

3 The rate of filling of the Ca^{2+} store in 2.1 mM-Ca^{2+}-containing solution was faster (time to half-maximal response, $t_{\frac{1}{2}} = 29 \pm 2.2$ s, $n = 4$) than the rate of depletion in Ca^{2+}-free solution ($t_{\frac{1}{2}} = 3 \pm 0.3$ min, $n = 3$). The gradual depletion of this store was much slower at 18°C than at 35°C, and in the presence of vanadate which is known to inhibit Ca^{2+}-ATPases.

4 Methoxyverapamil (D600, 10^{-6}–10^{-5} M) had no appreciable effect on the direct Ca^{2+} uptake or on the release of Ca^{2+} from the store by ACh and Ang II. Mn^{2+} (10^{-3} M) completely inhibited the direct pathway to the internal Ca^{2+} store and also reduced the release of Ca^{2+}.

5 ACh and Ang II induced repetitive depolarizations close to zero potential which did not parallel the transient contractions as a function of the time of perfusion in Ca^{2+}-free solution. Applications of 2 mM EGTA, 135 mM K^+ or Ca^{2+} antagonists which suppressed or reduced the drug-induced depolarizations did not affect appreciably the drug-induced contractions.

6 These results suggest that myometrial cells have an intracellular Ca^{2+} store sensitive to different stimulus substances. This store is not affected by depolarization of the plasma membrane and is certainly different from that described in voltage-clamp experiments.

Introduction

A rise in intracellular Ca^{2+} concentration is generally proposed as an activator of the contraction in skeletal muscle (Ebashi & Endo, 1968), cardiac (Katz, 1967) and smooth muscles (Filo *et al.*, 1965; Endo *et al.*, 1977). From results of experiments on voltage-clamped uterine smooth muscle, it has been suggested that there are two sources of intracellular ionized Ca^{2+}. One is the influx of Ca^{2+} from the outside through voltage-dependent channels, and the other is the release of Ca^{2+} from intracellular storage sites (Mironneau, 1973). The release of internal Ca^{2+} is also demonstrated by stimulating myometrial preparations in Ca^{2+}-free solution with different agonists such as acetylcholine and angiotensin II (Edman & Schild, 1962; Hamon & Worcel, 1979). The mechanism of action of stimulant substances in uterus include activation of different excitation-contraction coupling mechanisms: (i) an acceleration of action potential discharge or an increase in spike amplitude may increase the cytoplasmic Ca^{2+} concentration since it is believed that Ca^{2+} ions carry the inward current responsible for the upstroke (Mironneau, 1974; Vassort, 1975); (ii) a direct increase in Ca^{2+} permeability may be produced through receptor-operated channel activation (Bolton, 1979; Bolton & Kitamura, 1983); (iii) a release of intracellular Ca^{2+} may be induced from a limited store whose characteristics of exchange have been recently studied in intestinal (Hurwitz, 1975; Casteels & Raeymaekers, 1979; Brading & Sneddon, 1980) and vascular smooth muscles (Deth & Van Breemen, 1977; Droogmans *et al.*, 1977; Casteels & Droogmans, 1981; Haeusler *et al.*, 1981).

The purpose of the experiments described here

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was to investigate the characteristics of the intracellular Ca^{2+} store sensitive to acetylcholine and angiotensin II on rat myometrial preparations perfused in Ca^{2+}-free EGTA-containing solutions. Since it has been shown that the amplitude of the drug-induced contractions in Ca^{2+}-free solution can be taken as a measure of an internal Ca^{2+} store (Casteels & Raeymakers, 1979), we studied the effects of various solutions and Ca^{2+} inhibitors on the filling and emptying of this store. Electrical effects of both agonists were investigated in parallel with contractile modifications.

Methods

Experiments were performed on longitudinal muscle, free of both endometrium and circular muscle, isolated from pregnant rat uterus at the end of pregnancy (18–20 days). Strips (60–80 μm wide, 2–3 mm long) and short muscle segments (obtained by crushing the preparation under a grid of fine silver wires) were used for mechanical and electrical recordings respectively. After a stabilizing period (30–60 min) in the reference solution, the preparation was ready for experimental recordings.

Physiological solutions had the following composition: (a) Reference solution (mM): NaCl 130, KCl 5.6, $CaCl_2$ 2.1, $MgCl_2$ 0.24 and glucose 11. The solution was aerated with O_2 and was buffered by Tris-HCl (8.3 mM) at pH 7.4. (b) In Ca^{2+}-free solution, $CaCl_2$ was omitted and EGTA was added at various concentrations (0.5 to 2 mM). (c) High-K^+ solution was obtained by substituting NaCl for KCl in equimolar amounts. (d) Ouabain (10^{-4} M) and K^+-free solution (KCl was omitted) were used in order to inhibit the Na^+-K^+ pump activity (Taylor *et al.*, 1970). (e) Manganese chloride and D600 were used as inhibitors of the inward current (Hagiwara & Nakajima, 1966; Fleckenstein, 1977). (f) Sodium orthovanadate (Na_3Vo_4) was used as an inhibitor of Ca^{2+}-ATPases (Varecka & Carafoli, 1982).

Angiotensin II (Hypertensin) was obtained from Ciba. Acetylcholine, isoprenaline and sodium orthovanadate were obtained from Sigma.

Electrical activity was recorded with conventional microelectrodes filled with 3M-KCl (resistance 40–50 MΩ, tip potential less than 10 mV). Isometric contractions were recorded in an experimental chamber which consisted of an open-topped channel, 3 × 3 × 20 mm, connected at one end to a four way tap opening directly into the channel (Mironneau *et al.*, 1980). The solution entered the channel at a rate of 15 ml min^{-1}. The different solutions were maintained at 35°C by means of a heating bath and at 18°C (room temperature), so that rapid changes of temperature were possible. The myometrial strips were stimulated either electrically by single pulses (10 ms, 2–5 V) through platinum electrodes located on each side of the experimental chamber or by perfusion of stimulant substances.

Control contractions to 10 s applications of 10^{-4} M acetylcholine and 10^{-6} M angiotensin II were estab-

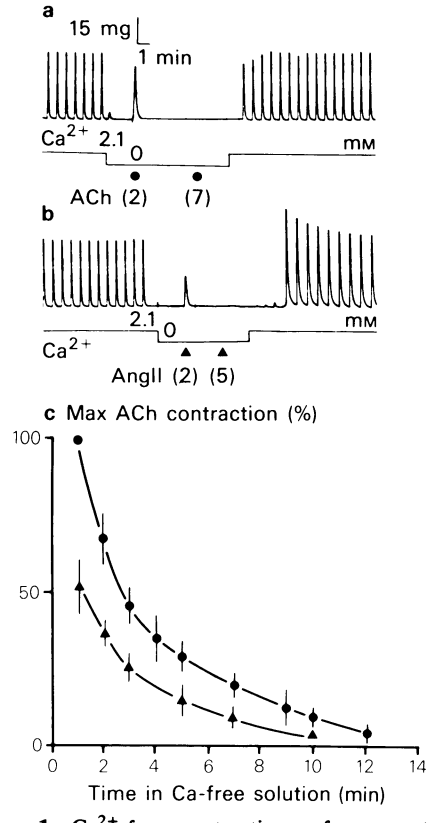

Figure 1 Ca^{2+}-free contractions of pregnant rat myometrium induced by supramaximal doses of acetylcholine (ACh) and angiotensin II (Ang II). (a) Twitch contractions triggered by electrical stimulation were rapidly suppressed by perfusing with a Ca^{2+}-free 0.5 mM-EGTA-containing solution. A transient contraction was elicited only once when 10^{-4} M ACh was added at different times of perfusion in Ca^{2+}-free solution, indicating in parentheses (min). (b) Angiotensin II (10^{-6} M) was applied after 2 and 5 min (numbers in parentheses) of perfusion in Ca^{2+}-free solution. Only one transient contraction was recorded. (c) The peaks of contractions elicited by 10^{-6} M Ang II (▲) and 10^{-4} M ACh (●) in Ca^{2+}-free solution are plotted against time of exposure to Ca^{2+}-free solutions preceding the addition of the drugs. They are expressed as a percentage of the maximal ACh-induced contraction obtained after 1 min of perfusion in Ca-free solution. ACh and Ang II were applied for 10 s. Each point represents the mean and vertical lines the s.e.mean of 5 preparations.

lished at the beginning of each experiment in normal physiological solution. The maximal contractions obtained after the addition of each drug were not significantly different (15 preparations).

Results

Contractile responses of uterine smooth muscle induced by acetylcholine and angiotensin II in Ca^{2+}-free solution

Isometric contractions induced by electrical stimulation as well as contractures induced by high-K^+ solutions were inhibited in a few seconds when the reference solution was replaced by a Ca^{2+}-free EGTA-containing solution. Edman & Schild (1962) demonstrated that uterus washed in Ca^{2+}-free solution could contract again in response to high concentrations of acetylcholine (ACh) suggesting the existence of an internal Ca^{2+} store. Figure 1a shows that supramaximal concentrations of ACh (10^{-4} M) applied for 10 s, evoked only one transient contraction in Ca^{2+}-free solution. When angiotensin II (Ang II, 10^{-6} M) was added independently of ACh (Figure 1b), the single contraction induced by Ang II was smaller than the ACh-induced response obtained after the same time of perfusion in Ca^{2+}-free solution. Readmitting reference solution to the bath after ACh application caused the return of normal triggered contractions within a few minutes. After Ang II application, increased twitch contractions were first recorded whose amplitude progressively returned to a normal value within 15 min (Figure 1b). Generally, 20 min of recovery in Ca^{2+}-containing solution were allowed between successive perfusions in Ca^{2+}-free solution. The size of the transient contractions induced by ACh and Ang II was largely dependent on the time interval between removal of Ca^{2+} and drug application. In Figure 1c, the contractions induced by ACh and Ang II were plotted against the time of perfusion in Ca^{2+}-free solution; their amplitudes were expressed as a percentage of the maximal ACh-induced contraction obtained after 1 min of perfusions in Ca^{2+}-free solution. These results show that both contractions decreased in a similar way as a function of time, and were lost within 10–12 min (5 preparations). The time at which the ACh-induced contraction was decreased by 50% was 3 ± 0.3 min (from 3 different curves). In Figure 2, the two agon-

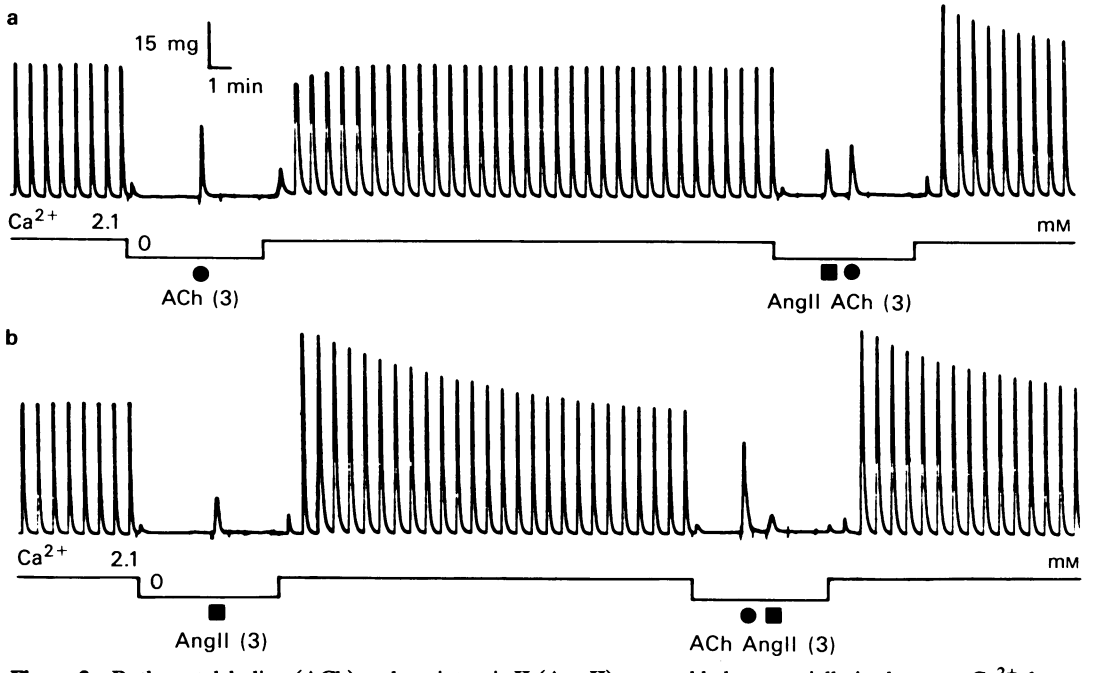

Figure 2 Both acetylcholine (ACh) and angiotensin II (Ang II) were added sequentially in the same Ca^{2+}-free solution. The test contractions were recorded after 3 min of perfusion in Ca^{2+}-free solution (number in parentheses). (a) A previous application of Ang II reduced the ACh-induced contraction by $34 \pm 5\%$ ($n = 5$) as compared to that obtained in the absence of Ang II. (b) After the development of the ACh-induced contraction, the response to Ang II was decreased by $66 \pm 8\%$ ($n = 3$) as compared to that obtained in the absence of ACh. Notice that, after Ang II application, return to the control solution induced increased twitch contractions which progressively returned to the normal value within 15 min.

ists were added sequentially during a Ca^{2+}-free perfusion (first Ang II, then ACh and *vice versa*). Each agent reduced the contraction subsequently induced by the other agonist and measured after the same time of perfusion in Ca^{2+}-free solution (3 min). It must be noted that control contractions to each agonist were obtained before and after every sequence. Consistent with its smaller effect on internal Ca^{2+}, Ang II was less effective in reducing the ACh-induced contraction ($34 \pm 5\%$, $n = 5$) than ACh in acting on the Ang II-induced contraction ($66 \pm 8\%$, $n = 3$).

Filling of the internal Ca^{2+} store sensitive to drugs

After complete disappearance of the drug-induced contraction in Ca^{2+}-free solution, uterine preparations were incubated in Ca^{2+}-containing solutions. This loading period was followed by a 2 min wash in Ca^{2+}-free EGTA-containing solution. Then, a supramaximal dose of ACh or Ang II was applied for 10 s in order to produce a transient contraction. Figure 3 shows the different ACh-induced contractions obtained after the same loading period in 2.1 mM-Ca^{2+} but for three different procedures: (a) no contraction, (b) twitch contractions triggered by 10 ms electrical stimulation every 35 s, (c) tetanus induced by trains of 10 ms pulses at a frequency over 1 Hz. It is obvious that the responses induced by ACh were largely dependent on the contractile state of the preparation during the Ca^{2+} loading period. The increase in ACh-induced contractions was $50 \pm 7\%$ ($n = 5$) when twitch contractions were triggered, and $100 \pm 15\%$ ($n = 4$) when fused tetani were generated. Therefore, in the following experiments, the incubation in Ca^{2+}-containing solution was carried out on quiescent preparations for different periods of time and with different external Ca^{2+} concentrations. In Figure 4a, the response to ACh was obtained on the same preparation after successive incubations in 2.1 and 0.2 mM Ca^{2+}. When the preparation was loaded in 0.2 mM Ca^{2+} for 5 min the amplitude of the ACh-induced contraction was strongly decreased in comparison to that obtained in 2.1 mM Ca^{2+}. In Figure

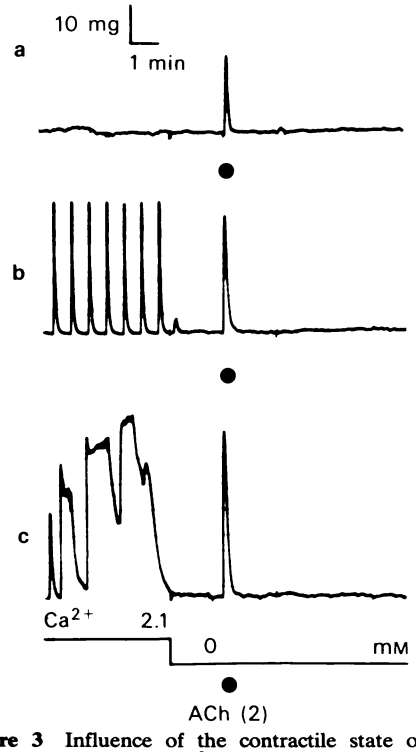

Figure 3 Influence of the contractile state of the uterine preparation in Ca^{2+}-containing solution on the amplitude of acetylcholine (ACh) -induced contractions obtained after 2 min of perfusion in Ca^{2+}-free solution (number in parentheses). (a) No contraction was triggered during Ca loading (5 min). Twitch contractions (b) or tetanus (c) were elicited by electrical stimulation.

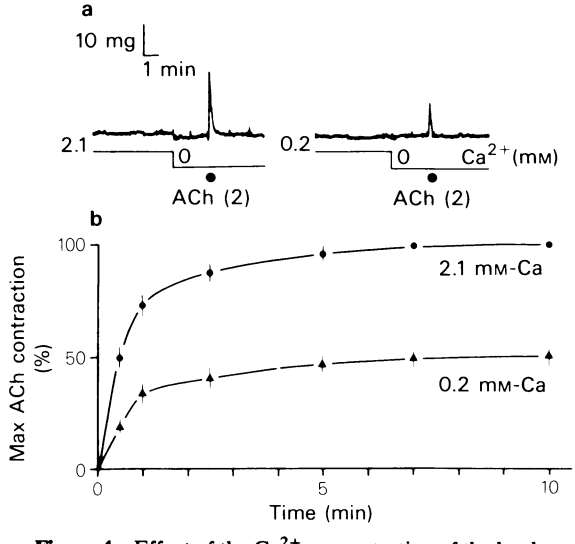

Figure 4 Effect of the Ca^{2+} concentration of the loading solution on the amplitude of transient contractions induced by acetylcholine (ACh) 10^{-4} M. The same Ca^{2+}-depleted preparation was successively loaded for 5 min in solutions of 2.1 or 0.2 mM Ca^{2+}. The response to ACh was measured after 2 min (in parentheses) of perfusion in Ca^{2+}-free solution. (b) The amplitude of ACh-induced contractions is plotted as a function of the loading time of Ca^{2+}-depleted preparations in solutions containing 2.1 mM (●) or 0.2 mM Ca^{2+} (▲). The tension is expressed as a percentage of the response obtained after a loading time of 10 min in a solution containing 2.1 mM Ca^{2+}. Each point represents the mean of 7 preparations; vertical lines show s.e.mean.

4b, the amplitude of the contractions is plotted as a function of the Ca^{2+} loading time for two different concentrations of Ca^{2+} (i.e. 2.1 and 0.2 mM). The contractions which were expressed as a percentage of the maximal response obtained after incubation in 2.1 mM Ca^{2+} (7 preparations) reached a plateau value after a loading period in Ca^{2+}-containing solution exceeding 5–7 min, and this plateau level depended on the external Ca^{2+} concentration. Moreover, it can be noted that after 29 ± 2.2 s (4 different curves) of loading in 2.1 mM Ca^{2+}, the contraction had reached 50% of its maximal value. The contractions obtained after short Ca^{2+} loading periods at 18°C were larger than those obtained after similar loading times at 35°C since readmission of Ca^{2+} at 18°C always induced a transient contraction. However, for longer periods of Ca^{2+}-loading (10 min or more) the influence of the Ca^{2+}-induced contraction at 18°C was greatly reduced or suppressed. The effects of Ca^{2+} antagonists such as D600 and manganese chloride were studied on the drug-induced contractions elicited after 2 min of perfusion in Ca^{2+}-free solution (Figure 5). Mn^{2+} ions (10^{-3} M) and

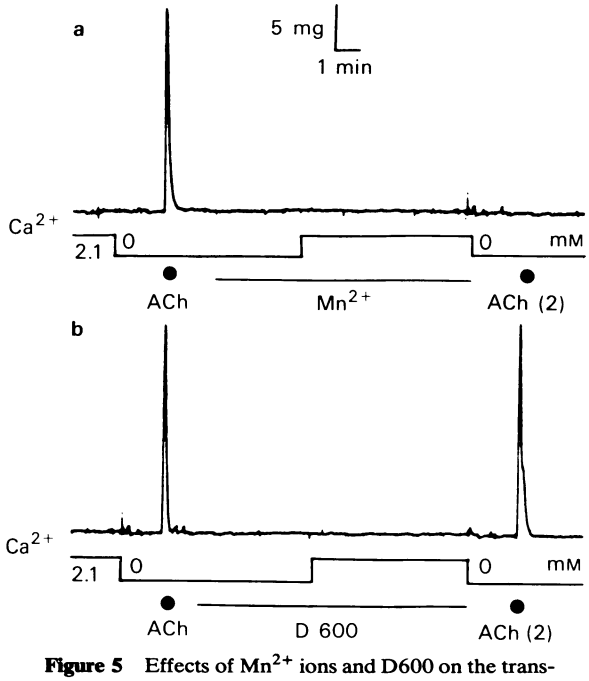

Figure 5 Effects of Mn^{2+} ions and D600 on the transient contractions induced by acetylcholine (ACh) 10^{-4} M in Ca^{2+}-free solutions. (a) When Mn^{2+} (10^{-3} M) was added during Ca^{2+} loading, the ACh-induced contraction was suppressed (5 preparations). (b) The response to ACh was not significantly modified after Ca^{2+} loading in the presence of 10^{-5} M D600 (8 preparations). In parentheses: times in Ca^{2+}-free solution (min).

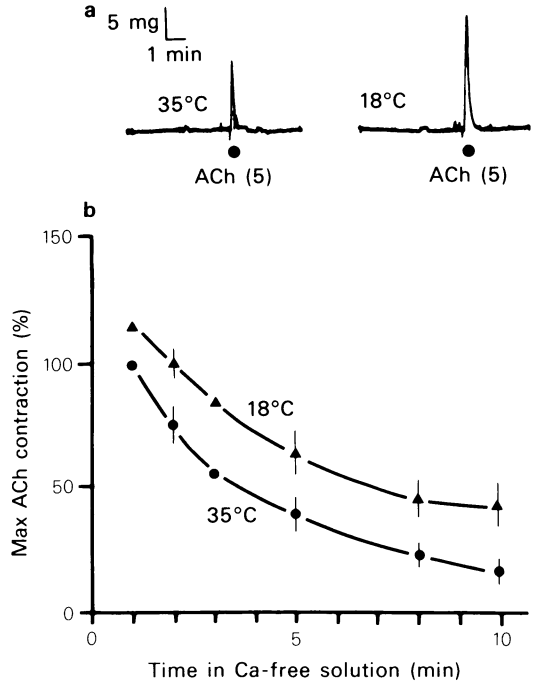

Figure 6 Effect of the temperature of the Ca^{2+}-free solution on the amplitude of contractions induced by acetylcholine (ACh) 10^{-4} M. Responses to ACh after exposing the preparation for 5 min (in parentheses) to a Ca^{2+}-free solution either at 35°C or 18°C. The Ca^{2+} loading was obtained by perfusing the preparation for 10 min with a solution containing 2.1 mM Ca^{2+} at 35°C. (b) The contractions elicited by 10^{-4} M ACh in Ca^{2+}-free solution are plotted against time of exposure to Ca^{2+}-free solutions either at 35°C (●) or 18°C (▲). The tension is expressed as a percentage of the contraction induced by ACh after an exposure of 1 min to a Ca^{2+}-free solution at 35°C. Points with vertical lines represent the mean and s.e.mean of 5 preparations. Other points correspond to an individual experiment.

D600 (10^{-5} M) were added 4–5 min before Ca^{2+} readmission and during Ca^{2+} loading. Mn^{2+} ions were very effective in blocking the ACh-induced contraction (98 ± 2%, $n = 5$) while D600 had no effect (2 ± 1%, $n = 8$). Similar results were obtained by using Ang II instead of ACh to induce a transient contraction in Ca^{2+}-free solution (7 preparations).

Depletion of the internal Ca^{2+} store sensitive to drugs

In the following experiments, the preparations were loaded for 10 min in 2.1 mM Ca^{2+}-containing solution at 35°C. Figure 6a shows the record of contractions induced by ACh after exposure of the preparation to a Ca^{2+}-free solution for 5 min either at 35°C

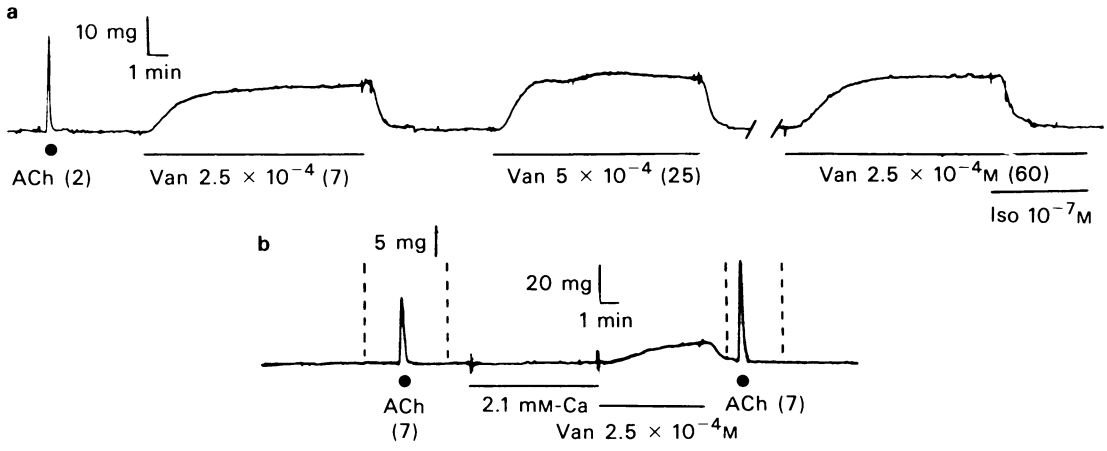

Figure 7 Effect of vanadate on preparations perfused with Ca^{2+}-free solutions. (a) After the transient contraction induced by acetylcholine (ACh) 10^{-4} M, repetitive contractions were obtained with vanadate (8 preparations). In parentheses: times in Ca^{2+}-free solution (min). Isoprenaline (Iso, 10^{-7} M) completely relaxed the vanadate-induced contraction. (b) ACh-induced contractions measured after 7 min of perfusion in Ca^{2+}-free solution with or without a preceding application of vanadate in Ca^{2+}-free solution. The contraction was increased by $50 \pm 8\%$ ($n = 5$) after vanadate application.

or at 18°C. After incubation at 18°C the ACh-induced contractions were larger than those obtained at 35°C. The different results for different incubation times in Ca^{2+}-free solution are represented in Figure 6b. For example, after 10 min of perfusion in Ca^{2+}-free solution at 18°C, the contraction was increased by 2.5 fold (5 preparations) when compared to that obtained at 35°C, suggesting that at 18°C a larger amount of Ca^{2+} was released from the internal store.

Vanadate has been described as a potent inhibitor of both Na^+-K^+-ATPase (Grover *et al.*, 1980) and Ca^{2+}-ATPase (Di Polo *et al.*, 1979; Varecka & Carafoli, 1982). During perfusion of uterine preparations with Ca^{2+}-free solutions, vanadate produced sustained contractions (8 preparations; Figure 7a). Moreover, repetitive vanadate-induced contractions were obtained whatever the time of perfusion in Ca^{2+}-free solutions. They were completely relaxed by isoprenaline (10^{-7} M, 5 preparations) or by washing out vanadate. Similar results were obtained when the preparations were continuously perfused with a K^+-free ouabain-containing solution in order to inhibit the Na^+-K^+ pump (3 preparations). The ACh-induced contraction was dependent on whether or not vanadate was added to the Ca^{2+}-free solution. As shown in Figure 7b, the ACh-induced contraction measured after 7 min of perfusion in Ca^{2+}-free solution was increased by $50 \pm 8\%$ ($n = 5$) when obtained after a vanadate-induced contraction.

The effects of methoxyverapamil (D600) and manganese chloride were studied on the drug-induced contractions elicited after 7 min of perfusion in Ca^{2+}-free solution (Figure 8). When D600 (10^{-6} or 10^{-5} M) was added at the beginning of the Ca^{2+}-free perfusion, no significant effect was observed on the ACh-induced contraction ($2 \pm 1\%$, $n = 5$). When manganese (10^{-3} M) was added under similar conditions, the amplitude of the contraction was reduced by $40 \pm 5\%$ ($n = 4$) indicating that Mn^{2+} ions may decrease the release of Ca^{2+} from its internal store. The sequence in which the responses were obtained did not interfere with the effects of the Ca^{2+} antagonists.

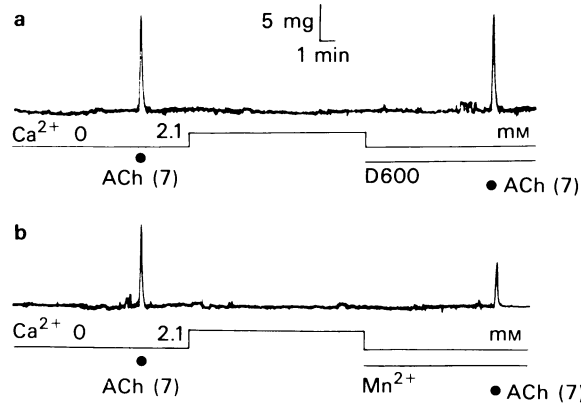

Figure 8 Effect of D600 and Mn^{2+} ions on the transient contractions induced by acetylcholine (ACh) 10^{-4} M in Ca^{2+}-free solutions. (a) When added at the beginning of the Ca^{2+}-free perfusion, D600 10^{-5} M had no significant effect (5 preparations). (b) Mn^{2+} 10^{-3} M reduced the response to ACh ($40 \pm 5\%$, $n = 4$). In parentheses: times in Ca^{2+}-free solution (min).

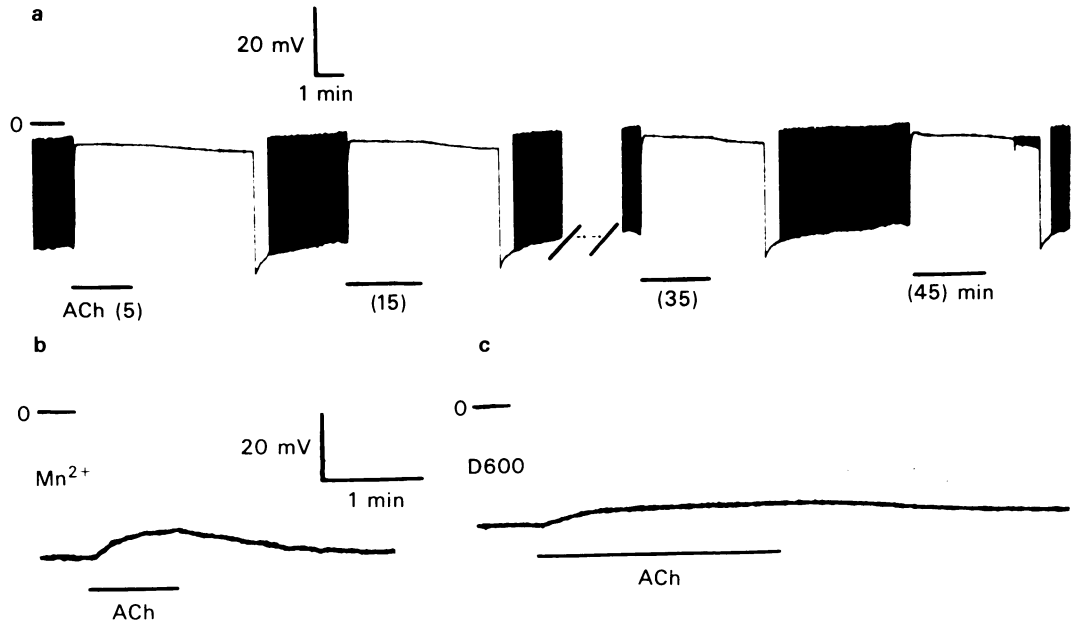

Figure 9 Effect of acetylcholine (ACh) 10^{-4} M on rhythmic electrical activity recorded intracellularly in Ca^{2+}-free solutions. (a) Repeated applications of ACh depolarized the membrane to -4.9 ± 0.6 mV ($n = 11$). In parentheses: times in Ca^{2+}-free solution (min). (b) Mn^{2+} ions (2.5×10^{-3} M) suppressed the electrical responses and repolarized the membrane to about -45 mV. ACh induced a small depolarization (7 ± 1.2 mV, $n = 4$). (c) D600 (10^{-6} M) inhibited rhythmic activity, the membrane remaining polarized at about -35 mV. ACh induced a limited depolarization (5 ± 0.7 mV, $n = 4$).

Influence of membrane potential on the internal Ca^{2+} store sensitive to drugs

In Ca^{2+}-free solution, treatment with concentrations of EGTA higher than 2 mM or with increased-K^+ solutions promptly depolarized the membrane to a steady state (about -5 mV) without the development of any action potential (Mironneau *et al.*, 1982). The transient ACh- or Ang II-induced contractions were not significantly reduced by membrane depolarizations generated by either 2 mM-EGTA or 135 mM-K^+ ($3 \pm 2\%$, $n = 6$).

Effects of acetylcholine and angiotensin II on membrane potential in Ca^{2+}-free solution

In Ca^{2+}-free solution (containing 0.5 mM EGTA) the uterine membrane was depolarized to about -35 mV. It has been shown that, under these conditions, spontaneous action potentials appeared which were dependent on a prolonged inward Na^+ current (Mironneau *et al.*, 1982). In Figure 9a, repetitive applications of ACh (10^{-4} M) resulted within 1 min in a rapid blockade of activity, the membrane remaining depolarized at a steady value (-4.9 ± 0.6 mV, $n = 11$). This sustained plateau was rapidly suppressed when ACh was removed from the solution and normal rhythmic activity reappeared. On the same preparation similar ACh-induced depolarizations were observed for 50–55 min in Ca^{2+}-free solution indicating no parallelism of the contractile and membrane responses against time. Ang II (10^{-6} M) also produced repetitive depolarizations of the uterine membrane (-5.2 ± 1.1 mV, $n = 5$). Mn^{2+} ions and D600 blocked the rhythmic action potentials when applied in Ca^{2+}-free solution. Mn^{2+} ions (2.5×10^{-3} M) induced a repolarization of the membrane to about -45 mV, while D600 (10^{-7}–10^{-6} M) had no effect on the resting potential which remained at about -35 mV (Mironneau *et al.*, 1982). In the presence of Ca^{2+} antagonists (Figure 9b), application of ACh or Ang II produced only a small depolarization (about 4–8 mV, in 8 different preparations). When Ca^{2+} antagonists were withdrawn from the Ca^{2+}-free solution, reference resting potentials returned within 1–2 min.

Discussion

The existence of an internal Ca^{2+} store that can be mobilized by excitatory drugs has been previously

proposed for the uterus (Edman & Schild, 1962; Sakai *et al.*, 1982) and other smooth muscles (Ohashi *et al.*, 1975; Droogmans *et al.*, 1977; Casteels & Raeymaekers, 1979; Brading & Sneddon, 1980). In Ca^{2+}-free solution, Casteels & Droogmans (1981) have shown the existence of a clear relationship between the rate of depletion of the internal Ca store and the rate of ^{45}Ca efflux from the cellular compartment. In analogy with this observation, we propose that in rat myometrium, a Ca^{2+} store can be emptied in Ca^{2+}-free EGTA-containing solution by the application of supramaximal doses of both ACh and Ang II assuming that changes of the cytoplasmic Ca^{2+} concentration during stimulation can be estimated by following the contraction development. The amount of Ca^{2+} in the intracellular store, as measured from the change in contraction amplitude to ACh or Ang II in Ca^{2+}-free solution, decreased as a function of time indicating that the store was completely lost after 10–12 min of perfusion in Ca^{2+}-free solution at 35°C. Three lines of evidence indicate a partial cross-tachyphylaxis between ACh and Ang II: (i) each agonist reduced the contraction elicited by the other during the same Ca^{2+}-free perfusion; (ii) both contractions decreased at a similar rate against time of perfusion in Ca^{2+}-free solution; (iii) both contractions had a similar sensitivity to temperature, Ca^{2+} antagonists and membrane potential. It is beyond the scope of this paper to speculate on how a common Ca^{2+} source could be released by different receptor interactions. However, in intestinal smooth muscle Bolton *et al.* (1981) have proposed different models to account for the observation that common ion channels can be opened by the activation of different membrane receptors. One of them invokes the presence of a diffusible substance (calcium) which would link activated receptors to the opening of ion channels. Similarly, release of Ca^{2+} from the association with receptors by the action of both ACh and Ang II may trigger an amplified release of Ca^{2+} from the internal store and the development of transient contractions. The disparity between the amplitudes of ACh- and Ang II-induced contractions could be dependent on the fact that, in visceral smooth muscles, Ang II receptors were fewer in number than muscarinic receptors (Yoshida *et al.*, 1979; Schirar *et al.*, 1980). The importance of a pharmacomechanical coupling mechanism is further suggested by: (i) the generation of similar ACh- and Ang II-induced contractions in polarized and depolarized preparations; (ii) the absence of relation between membrane depolarization and transient contraction induced by both ACh and Ang II as a function of time of perfusion in Ca^{2+}-free solution; (iii) the larger reduction of membrane depolarizations than that of contractions induced by Ca^{2+} antagonists during the application of each drug.

In Ca^{2+}-free solution, the amount of Ca^{2+} which activated the contractile proteins was not only determined by the amount which was released from the internal store, but also by both extrusion and sequestration of Ca^{2+} occurring simultaneously with the Ca^{2+} release. For example, decreasing the temperature of the perfusing Ca^{2+}-free solution from 35°C to 18°C had a clear effect on the gradual depletion of the Ca^{2+} store since the tension induced by ACh and Ang II was larger during depletion at 18°C. This observation is in good agreement with the idea that temperature decreased the Ca^{2+} extrusion, and consequently allowed a larger amount of Ca^{2+} to be released by ACh and Ang II. Similar results have been described in rabbit ear artery in response to noradrenaline application (Droogmans & Casteels, 1981). This suggestion was confirmed by using vanadate in order to inhibit Ca^{2+}-ATPases (Varecka & Carafoli, 1982). Thus, the drug-sensitive Ca^{2+} store appeared to be less depleted when vanadate was previously added in Ca^{2+}-free solution. The fact that vanadate-induced contractions were observed up to 1 h in Ca^{2+}-free solution suggest that Ca^{2+} ions were released from a cellular compartment (different from the ACh-sensitive Ca^{2+} store) which normally accumulated Ca^{2+} through ATP-dependent mechanisms. The relaxing effect of isoprenaline confirms the Ca^{2+} dependence of vanadate-induced contractions since isoprenaline may stimulate both Ca^{2+} sequestration and Ca^{2+} extrusion in smooth muscles (Casteels & Raeymaekers, 1979; Bülbring & Den Hertog, 1980).

The filling of the internal Ca^{2+} store in uterus appeared to be dependent on both external and cytoplasmic Ca^{2+} concentrations for the following reasons: (i) in quiescent Ca^{2+} preparations, the amount of Ca^{2+} accumulated in the internal store was noticeably decreased as the external Ca^{2+} concentration was reduced from 2.1 to 0.2 mM; (ii) decreasing the temperature of the Ca^{2+} loading solution to 18°C had a limited effect on the filling of the Ca^{2+} store; (iii) the rate of filling was faster than the rate of depletion in Ca^{2+}-free solution; (iv) during fused tetanus, a greater Ca^{2+} influx increased the cytoplasmic Ca^{2+} level and two fold more Ca^{2+} accumulated in the internal store. From these results and from previous suggestions made by Brading *et al.* (1980) and Casteels & Droogmans (1981), the existence of two pathways for Ca^{2+} uptake into the internal Ca^{2+} store can also be proposed for the uterus: the first consisting of a direct penetration of Ca^{2+} into the store which essentially depends on a passive diffusion, and a second, resulting from an increase in cytoplasmic Ca^{2+} level which depends on increased Ca^{2+} influxes through voltage- or/and receptor-operated channels.

Ca^{2+} antagonists such as D600 and manganese

chloride exerted various effects on both direct Ca^{2+} uptake and Ca^{2+} release from the internal store. D600 had no inhibitory action on both Ca^{2+} movements. In contrast, manganese completely inhibited the direct Ca^{2+} uptake and strongly reduced (about 40%) the release of Ca^{2+} induced by ACh and Ang II in Ca^{2+}-free solution. Probably, Mn^{2+} ions competed with Ca^{2+} ions in the surface membrane and thus suppressed most of the membrane Ca^{2+} movements. Most of our findings support the hypothesis that the drug-sensitive Ca^{2+} store may be located in the close vicinity of the surface membrane. It may be suggested that the peripheral sarcoplasmic reticulum which has been described in close association with the plasma membrane (Devine *et al.*, 1972; Rubanyi *et al.*, 1980) would represent the drug-sensitive Ca^{2+} store.

In conclusion, ACh and Ang II may release Ca^{2+} from a limited intracellular store in myometrial cells which can be filled by Ca^{2+} ions proceeding from both external and cytoplasmic Ca^{2+} compartments. The mechanism of action of the two stimulant substances seems to depend essentially on pharmacomechanical coupling since membrane depolarization may not be related to transient contractile responses in Ca^{2+}-free solutions. A fundamental characterization of this internal Ca^{2+} store will require more knowledge about the biochemical regulations of subcellular membrane fractions.

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