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The spasmogenic action of oxytocin in the rat uterus – comparison with other agonists

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1 A low concentration (0.2 nM) of oxytocin induced phasic tension development in the isolated uterus of the day-22 pregnant rat. Tonic spasm was also induced by higher concentrations of oxytocin (2 and 20 nM). Spasmogenic responses to bradykinin and potassium chloride (KCl) also contained phasic and tonic components while acetylcholine induced tonic spasm only.

2 The phasic component of the responses to oxytocin and to bradykinin and both components of the response to KCl were inhibited by (+)-*cis* diltiazem (0.1 and 1 μM). The tonic component of the responses to oxytocin and to bradykinin and the responses to acetylcholine were only reduced by (+)-*cis* diltiazem at concentrations $>10\,\mu$M.

3 (−)-*cis* Diltiazem was less potent than (+)-*cis* diltiazem as an inhibitor of calcium (Ca^{2+})-induced spasm in a depolarizing medium and of the phasic spasms induced by oxytocin. The two isomers were of similar potency as inhibitors of oxytocin-induced tonic spasm.

4 Spasmogenic responses to oxytocin, bradykinin, acetylcholine and KCl were decreased when uteri were bathed in media which were Ca^{2+}-free or of low Na^+ content. However, there was no correlation between the rank order of sensitivity of the four spasmogens to the changed media and to their inhibition by (+)-*cis* diltiazem.

5 Oxytocin (0.2 nM) increased the frequency, duration and amplitude of spike activity, measured by extracellular electrical recording, in parallel with enhancement of phasic tension development. With higher concentrations of oxytocin (2 and 20 nM) spike firing was initially continuous but often subsequently ceased despite the associated tonic contracture. After incubation in (+)-*cis* diltiazem (10 μM), oxytocin (0.2, 2 and 20 nM) produced graded tonic spasm without spike activity.

6 Oxytocin (0.2 nM) produced a small increase in $^{45}Ca^{2+}$ influx into myometrium as assessed by the 'lanthanum method'. Higher concentrations of oxytocin (2 and 20 nM) did not increase $^{45}Ca^{2+}$ influx.

7 It is concluded that the phasic component of the response of the uterus to oxytocin and bradykinin is associated with Ca^{2+} influx via voltage-dependent Ca^{2+} channels. The tonic component is due to another mechanism(s) which does not appear to involve Ca^{2+} influx. All of the spasmogenic response to KCl can be explained by Ca^{2+} influx through voltage-dependent Ca^{2+} channels. These channels do not appear to be involved in the spasmogenic response to acetylcholine.

Introduction

Oxytocin is a potent and selective spasmogen of uterine smooth muscle, an action which is physiologically relevant at parturition (Fuchs, 1978).

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Incubation of the isolated uterus of the mouse or rat with a low concentration of oxytocin enhances the force, frequency and duration of spontaneous phasic tension development accompanied by an increase in spike frequency and train duration (Marshall, 1968; Suzuki & Kuriyama, 1975). Higher concentrations of oxytocin produce a prolonged contracture of the muscle and a sustained depolarization after an initial increase in spike frequency.

The mechanisms linking interaction of oxytocin

with its receptor and the observed mechanical and electrical changes are poorly understood. The role of specific cations and the relative importance of their extracellular and intracellular origins is particularly unclear. Marshall (1963, 1968) observed that lowering the extracellular sodium ion (Na^+) and calcium ion (Ca^{2+}) concentrations markedly reduced the spasmogenic response of the rat uterus to oxytocin. Mironneau (1976) showed that low concentrations of oxytocin increased an inward ionic current associated with a depolarizing voltage step and proposed that oxytocin opens ion channels, particularly for Ca^{2+}, in the plasma membrane. Phasic tension development induced in the uterus of the term pregnant rat by low concentrations of oxytocin is very sensitive to inhibition by calcium entry blockers (Hollingsworth *et al.*, 1983; Granger *et al.*, 1985a), suggesting that this response involves an increase in Ca^{2+} influx through the voltage-operated channels described by Bolton (1979).

Intracellular mechanisms may also contribute to the action of oxytocin as the drug can inhibit the binding of Ca^{2+} to myometrial microsomes (Carsten, 1974). Recently Sakai *et al.* (1981, 1982) and Ashoori *et al.* (1985) have suggested that a small component of the response of the non-pregnant rat uterus to oxytocin may not involve Ca^{2+}-dependent mechanisms.

In the present experiments, we have examined the mechanism of the spasmogenic action of oxytocin using tissue bath experiments, extracellular electrical recording and $^{45}Ca^{2+}$ influx measurements. Studies have also been performed with KCl, bradykinin and acetylcholine to determine whether their spasmogenic action is similar to that of oxytocin. Results of some of these experiments have been communicated to the British Pharmacological Society (Granger *et al.*, 1985b; Edwards *et al.*, 1986).

Methods

Uteri were obtained from day-22 pregnant Sprague-Dawley rats (250–350 g) supplied by the Manchester University Animal Unit and killed before 10 h 00 min. Uterine horns were freed of foetuses and placentae and placed in a physiological salt solution (PSS) at room temperature. All experiments were performed on longitudinal strips of whole uterus, except for measurements of $^{45}Ca^{2+}$ influx where myometrial strips (Granger *et al.*, 1986) were used.

Tissue bath experiments

To assess the effect of Ca^{2+}-free PSS on responses to spasmogens the uterus was initially bathed in normal PSS and exposed to maximally-effective concentrations of oxytocin (20 nM), acetylcholine (100 μM),

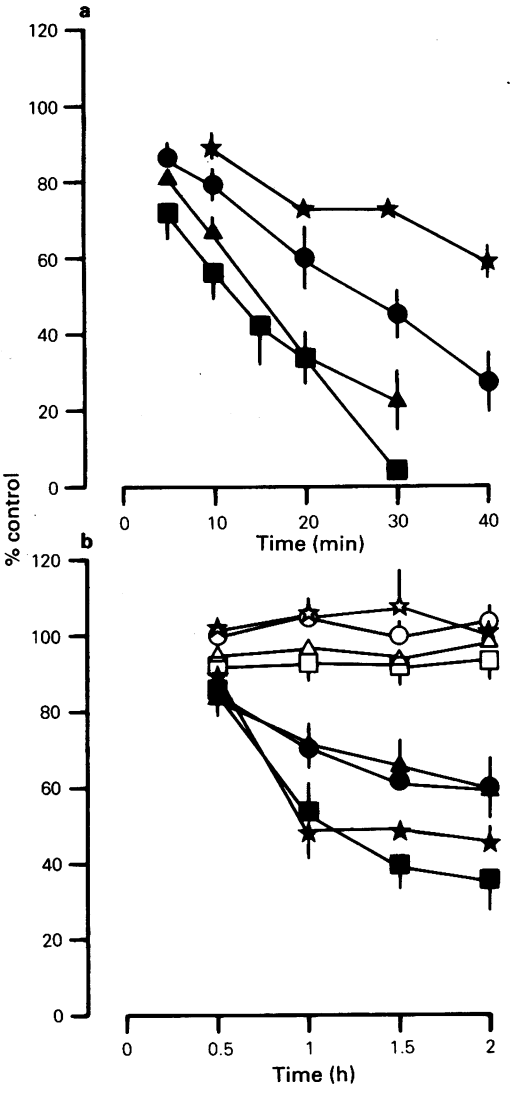

Figure 1 The effects of (a) a Ca^{2+}-free PSS containing 0.5 mM EGTA or (b) a low-Na^+ PSS on responses of isolated uterus of the term pregnant rat to oxytocin (20 nM; ■), acetylcholine (100 μM; ●), bradykinin (1 μM; ★) or KCl (40 mM; ▲). The ordinate scale represents the response expressed as a percentage of that initially obtained in normal PSS. The abcissae represent the subsequent duration of incubation in modified PSS (test tissues) or normal PSS (controls). In (b) the open symbols represent responses obtained in concurrent control tissues maintained in normal PSS. Note that, in normal PSS, there was no tendency for responses to any of the agonists to decrease with time. The points are the means and the vertical lines show the s.e.mean ($n = 5–8$).

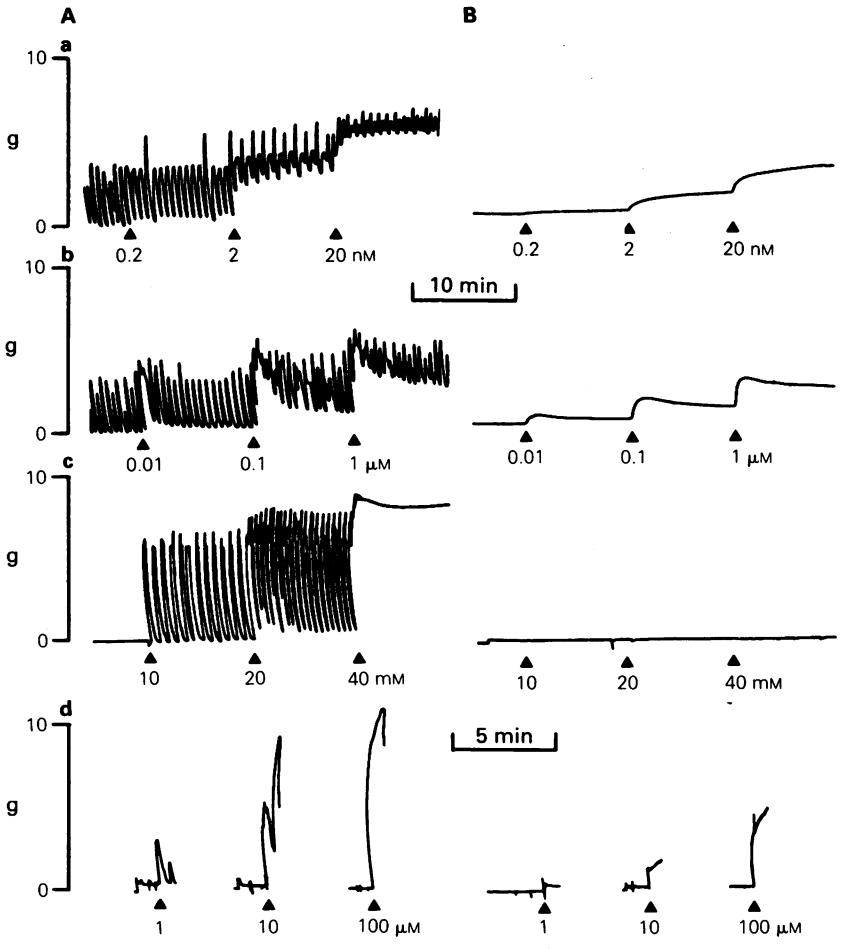

Figure 2 Tension development of isolated uterus of the term pregnant rat induced by (a) oxytocin, (b) bradykinin, (c) KCl or (d) acetylcholine. Responses were obtained (A) in normal PSS and (B) in the same tissues from 40 min after the commencement of incubation in PSS containing (+)-*cis* diltiazem (10 μM). The 10 min scale refers to (a), (b) and (c), the 5 min scale to (d).

bradykinin (1 μM) or KCl (40 mM) for 1 min every 20 min. Tissues were then incubated in a Ca^{2+}-free PSS (containing ethyleneglycol-bis-(β-amino-ethyl ether) N,N'-tetra-acetic acid, EGTA, 0.5 mM and no added Ca^{2+}) for varying times before spasmogen challenge was repeated. The Ca^{2+}-free PSS was then replaced with normal PSS and the spasmogen challenge repeated. The cycle of Ca^{2+}-free PSS and normal PSS was performed several times.

To assess the effects of low-Na^+ PSS (see below), the uterus was initially bathed in normal PSS and exposed to oxytocin (20 nM), acetylcholine (100 μM), bradykinin (1 μM) or KCl (40 mM) for 1 min every 30 min until constant responses were obtained. The bathing medium was changed to a low-Na^+ PSS and spasmogen challenge was then repeated every 30 min for up to 2 h with tissues incubated in the low-Na^+ PSS.

The rate of decline of the response in Ca^{2+}-free or low-Na^+ PSS was assessed as the regression of the response as a % of the response in normal PSS against time by the least squares method.

The effects of diltiazem were assessed against concentration-effect curves for the spasmogens in tissues bathed by normal PSS. The uterus was exposed to oxytocin, KCl, bradykinin or calcium chloride and their concentrations increased in a cumulative manner every 10 min. Mechanical responses were measured as the integrated tension (Granger *et al.*, 1986). Tissues were exposed to acetylcholine for 1 min every 5 min

and responses assessed as peak tension attained. These concentration-effect curves were constructed before and 40 min after incubation of tissues with increasing concentrations of (+)-*cis* or (−)-*cis* diltiazem.

Extracellular electrical recording

Simultaneous measurement of tension development and extracellular electrical activity was performed using the technique of Golenhofen & von Loh (1970). Strips of uterus were superfused with normal PSS and oxytocin (0.2, 2 and 20 nM) added cumulatively at 10 min intervals. Strips were then superfused with normal PSS (controls) or PSS containing (+)-*cis* diltiazem (10 μM) for 40 min before the oxytocin challenge was repeated in the continuing presence of diltiazem.

Lanthanum-resistant ^{45}calcium fraction

These experiments were performed according to the method of Granger *et al.* (1986). Myometrial strips were incubated in MOPS-buffered PSS containing $^{45}Ca^{2+}$ (250 nCi ml^{-1}) for 5 min and then transferred to MOPS-buffered PSS containing $^{45}Ca^{2+}$ with or without oxytocin (0.2, 2 or 20 nM) for 10 min. The tissues were subsequently washed in a Ca^{2+}-free MOPS-buffered PSS containing lanthanum chloride (10 mM) at 0°C for 120 min. The lanthanum-resistant $^{45}Ca^{2+}$ fraction was assessed as the ratio of tissue to medium contents (nCi g^{-1} of tissue: nCi ml^{-1} of medium).

Statistical analysis/drugs

The concentration of diltiazem (as $-\log_{10}$M) required to produce 50% inhibition (IC_{50}) of the response to a spasmogen was assessed as the mean ± s.e.mean by the method of Granger *et al.* (1985a). The significance of differences between means was assessed by Student's *t* test or by analysis of variance and the least significant difference test. Two-tailed statistical tests were used throughout.

The composition of normal PSS, the K^+-rich depolarizing PSS and the MOPS-buffered PSS are the same as those quoted by Granger *et al.* (1986). The composition of the low-Na^+ PSS (Na^+ content 17% of normal PSS) was (mM): Na^+ 25, K^+ 5.9, Ca^{2+} 2.55, Mg^{2+} 1.2, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, Cl^- 10, HCO_3^- 25, glucose 11 and sucrose 217. These solutions were isosmolar.

The following substances were used: (+)-*cis* and (−)-*cis* diltiazem hydrochloride (Synthelabo), oxytocin (grade X, Sigma), acetylcholine chloride (Sigma), bradykinin triacetate (Sigma), 3-(N-morpholino)-propanesulphonic acid (MOPS; BDH), EGTA (Sigma), lanthanum chloride (BDH) and sucrose (BDH). $^{45}Ca^{2+}$ (10–40 mCi mg^{-1}) was obtained as an aqueous solution of calcium chloride from Amersham International.

Results

Tissue bath experiments

Nature of agonist-induced mechanical responses Oxytocin (0.2 nM) produced an increase in the amplitude and frequency of phasic tension waves while oxytocin (20 nM) usually initiated maintained (tonic) mechanical responses with superimposed small phasic tension waves (Figure 2a). The response to oxytocin (2 nM) consisted of a variable proportion of the phasic and tonic components. Bradykinin (10 nM, 100 nM and 1 μM; Figure 2b) and KCl (10, 20 and 40 mM; Figure 2c) produced a mixture of phasic and tonic responses similar to those of oxytocin. Acetylcholine (1 to 100 μM; Figure 2d) induced a concentration-dependent monophasic or tonic contracture.

Control experiments (Figures 1, 3–5) showed that the effects of oxytocin, bradykinin, acetylcholine and KCl did not significantly change with time.

Effects of Ca^{2+}-free PSS and low Na^+ PSS When uteri were incubated in a Ca^{2+}-free PSS, responses to oxytocin (20 nM), acetylcholine (100 μM), bradykinin (1 μM) and KCl (40 mM) declined with time at an approximately constant rate (Figure 1a). There were differences between the rates of decline of responses (% min^{-1}) to KCl (−2.77 ± 0.64; mean ± s.e.mean, $n = 6$), oxytocin (−2.44 ± 0.29; $n = 6$), acetylcholine (−1.75 ± 0.15; $n = 6$) and bradykinin (−0.89 ± 0.11; $n = 8$) ($P = 0.002$, 1 factor analysis of variance). Responses to KCl declined faster than those to acetylcholine ($P < 0.05$) or bradykinin ($P < 0.01$) and responses to oxytocin declined faster than those to bradykinin ($P < 0.01$). Similarly when strips of uterus were incubated in a low-Na^+ PSS, responses to all spasmogens declined over 2 h to between 36 and 60% of those observed previously in normal PSS (Figure 1b). There were no differences between the rates of decline of responses (% h^{-1}) to oxytocin (−32.4 ± 5.8; $n = 8$), bradykinin (−18.4 ± 2.5; $n = 4$), acetylcholine (−16.9 ± 5.4; $n = 7$) or KCl (−15.4 ± 3.9; $n = 6$) ($P > 0.05$, 1 factor analysis of variance).

Effects of diltiazem The (+)-*cis* enantiomer of diltiazem is a selective calcium entry blocker (Flaim, 1984; Spedding, 1985; Granger *et al.*, 1986) and was used to identify those components of the mechanical responses to the agonists which were associated with Ca^{2+} influx through voltage-operated Ca^{2+} channels. The phasic component of tension development to

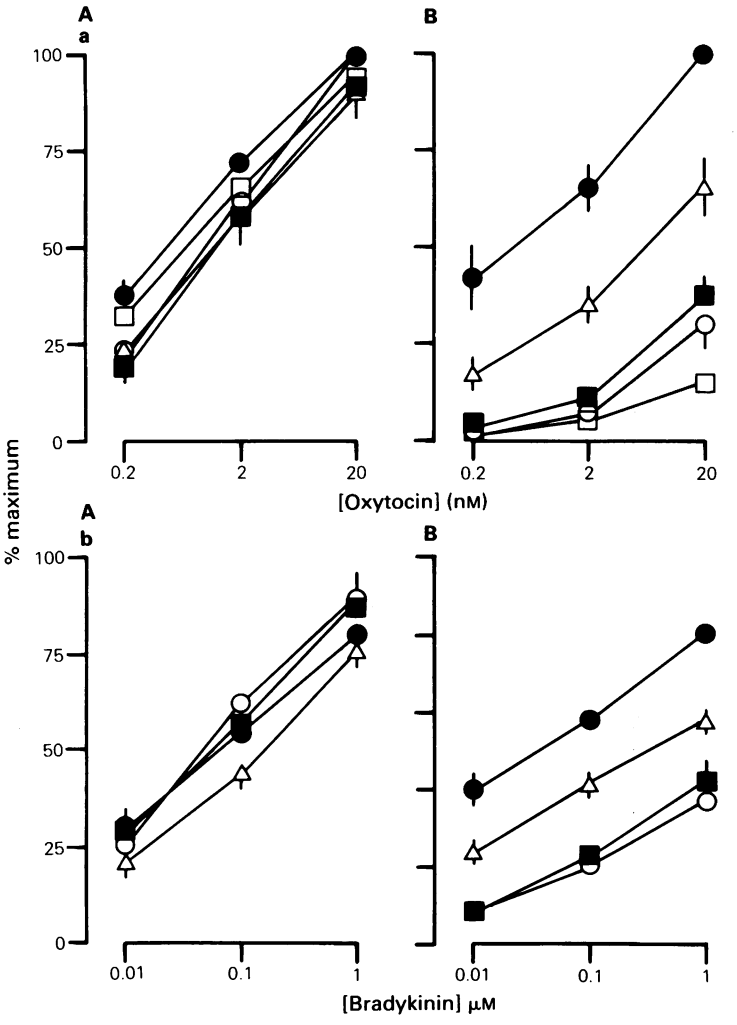

Figure 3 The effect of (+)-*cis* diltiazem on concentration-effect curves to (a) oxytocin or (b) bradykinin in the uterus of the term pregnant rat. The ordinates are the responses expressed as a percentage of the response to oxytocin (20 nM) or bradykinin (1 μM) in initial curves. The abcissae are the concentrations of agonists on a log scale. Shown are initial curves (●) and after 40 min incubation with (+)-*cis* diltiazem (B; △, 0.1 μM; ■, 1 μM; ○, 10 μM; □, 100 μM) or in respective time-matched concurrent controls (A). The points are the means and the vertical lines show the s.e.means ($n = 3-7$).

oxytocin (0.2, 2 or 20 nM) was abolished by (+)-*cis* diltiazem (1 μM) while higher concentrations of this enantiomer (10 and 100 μM) were needed to reduce the tonic component significantly (Figures 2a and 3a). Similarly the phasic component of the response to bradykinin was also more sensitive to inhibition by (+)-*cis* diltiazem than was the tonic component (Figures 2b and 3b). The mean $-\log_{10}$M $IC_{50} \pm$ s.e.mean for (+)-*cis* diltiazem versus oxytocin (0.2 nM) was 6.68 ± 0.13 ($n = 5$) and 7.67 ± 0.40 ($n = 3$) versus bradykinin (10 nM). (+)-*cis* Diltiazem was a potent inhibitor of the tonic component as well as the phasic component of the mechanical response to KCl (Figures 2c and 4a). There was no difference ($P > 0.05$) between the IC_{50} values for (+)-*cis* diltiazem versus KCl (10 mM and 40 mM) (6.80 ± 0.33 and 6.50 ± 0.15, respectively; mean $-\log_{10}$M $IC_{50} \pm$ s.e.mean, $n = 6$). By contrast, (+)-*cis* diltiazem was of low potency as an inhibitor of tension development evoked by acetylcholine (1 and 100 μM) for

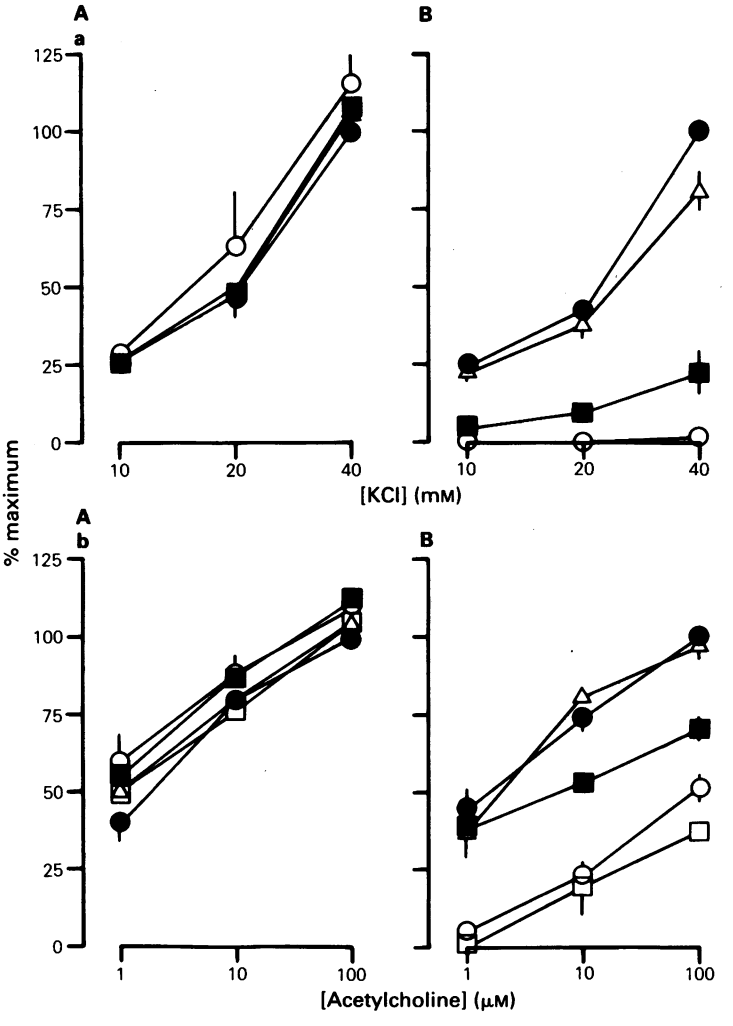

Figure 4 The effect of (+)-*cis* diltiazem on concentration-effect curves to (a) KCl or (b) acetylcholine in the uterus of the term pregnant rat. The ordinates are the responses expressed as a percentage of the response to KCl (40 mM) or acetylcholine (100 μM) in initial curves. The abcissae are the concentrations of agonists on a log scale. Shown are initial curves (●) and after 40 min incubation with (+)-*cis* diltiazem (B; △, 0.1 μM; ■, 1 μM; ○, 10 μM; □, 100 μM) or in respective time-matched concurrent controls (A). The points are the means and the vertical lines show the s.e.mean ($n = 6$–8).

which its IC_{50} values were respectively 5.82 ± 0.09 and 4.77 ± 0.20 (mean − $\log_{10}$M IC_{50} ± s.e.mean, $n = 6$ to 8; Figure 4b).

The inhibition of the tonic component of the tension response to oxytocin (20 nM) by high concentrations of (+)-*cis* diltiazem could be due to calcium entry blockade or another action. Since *cis*-diltiazem has been shown to exhibit stereospecificity as an inhibitor of KCl spasms of taenia coli (Nagao *et al.*, 1972), the effects of the (−)-*cis* enantiomer of diltiazem were examined in the present study. The ability of (−)-*cis* diltiazem to inhibit Ca^{2+} influx was assessed by measuring the inhibition of Ca^{2+}-induced spasm of uterus in a depolarizing PSS. (−)-*cis*-Diltiazem produced concentration-related inhibition of Ca^{2+}-induced spasm (Figure 5) but only at concentrations (10 μM and 100 μM) which were 100 times greater than those required of the (+)-*cis* enantiomer to produce similar antagonism (Granger *et al.*, 1986). High concentrations of (−)-*cis* diltiazem (10 and 100 μM) were

needed to inhibit both phasic and tonic spasms induced by oxytocin (Figure 5b).

Extracellular electrical recording

Oxytocin (0.2 nM) increased the frequency, and often the duration and amplitude, of spike discharges. These electrical events were associated with an increased frequency and amplitude of phasic tension waves (Figure 6a). Oxytocin (2 and 20 nM) initially produced continuous spike discharges which were associated with a tonic contracture of the uterus and superimposed phasic tension waves (Figure 6a). During the 10 min exposure to oxytocin (20 nM), the electrical discharges often became discontinuous and only associated with the phasic tension waves. Alternatively they ceased completely. After 40 min superfusion with (+)-*cis* diltiazem (10 μM), oxytocin (0.2 to 20 nM) produced a concentration-related slow rise in tone without phasic tension waves and without associated spike discharges (Figure 6b).

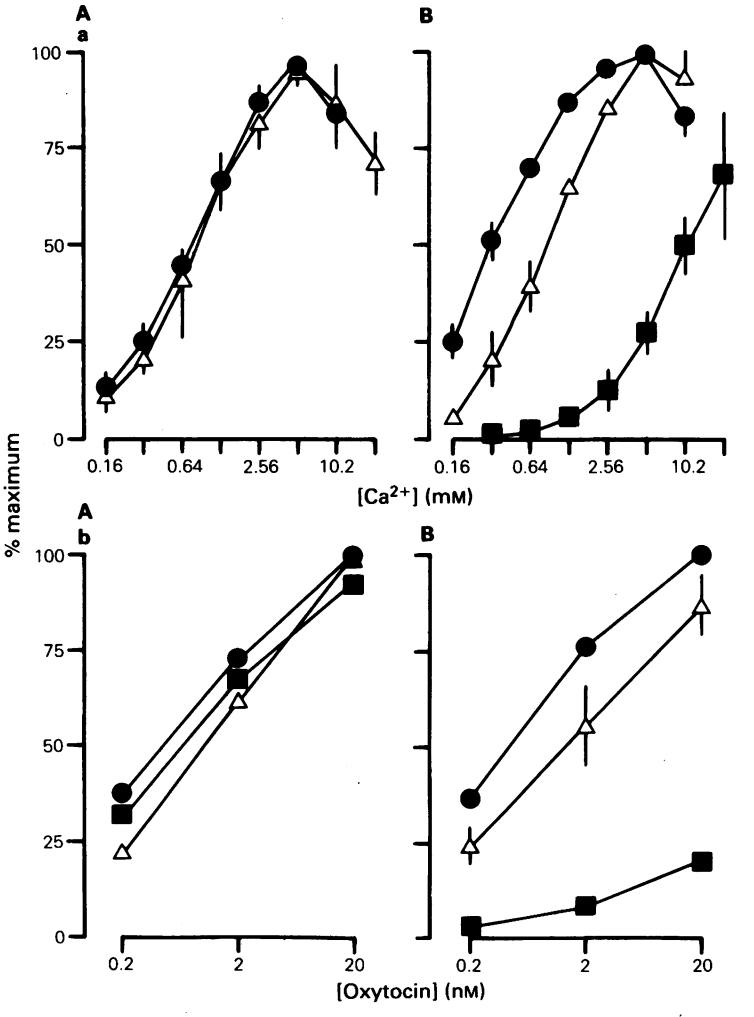

Figure 5 The effect of (−)-*cis* diltiazem on concentration-effect curves to (a) Ca^{2+} in a depolarizing MOPS-buffered PSS or (b) oxytocin. The ordinates are the responses expressed as a percentage of the response to Ca^{2+} (5.12 mM) or oxytocin (20 nM) in initial curves. The abcissae are the concentrations of agonists on a log scale. Shown are initial curves (●) and after 1 h incubation with (−)-*cis* diltiazem (B; △, 10 μM; ■, 100 μM) or in respective time-matched concurrent controls (A). The points are the means and the vertical lines show the s.e.mean ($n = 3-6$).

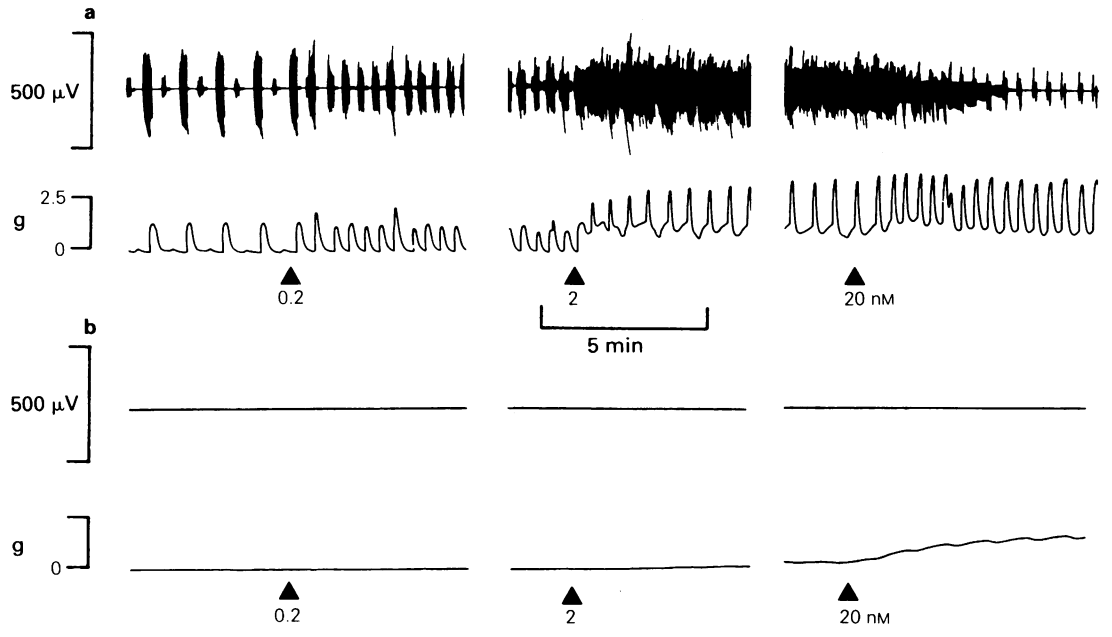

Figure 6 Effect of oxytocin on the extracellularly-recorded electrical (upper traces) and mechanical (lower traces) activity of isolated uterus from the term pregnant rat. Recordings are from the same tissue bathed in (a) normal PSS and (b) 40 min after the commencement of superfusion with PSS containing (+)-*cis* diltiazem (10 μM). Note the ability of (+)-*cis* diltiazem to abolish spontaneous and oxytocin-induced spike discharges and phasic tension waves but to leave tonic tension development unaffected.

Lanthanum-resistant $^{45}Ca^{2+}$ fraction

Incubation of myometrial strips with oxytocin (0.2 nM) for 10 min produced a small (18%) but significant ($P < 0.05$, 1 factor analysis of variance) increase in the lanthanum-resistant $^{45}Ca^{2+}$ fraction compared with controls (0.23 ± 0.01 ml g^{-1}, $n = 16$ and 0.19 ± 0.01 ml g^{-1}, $n = 16$, respectively). Incubation of myometrial strips with oxytocin (2 and 20 nM) produced no changes in the lanthanum-resistant $^{45}Ca^{2+}$ fraction (0.22 ± 0.01 ml g^{-1}, $n = 16$ and 0.22 ± 0.01 ml g^{-1}, $n = 16$, respectively) compared with controls.

Discussion

The present results demonstrate that the spasmogenic response of the rat isolated uterus to oxytocin consists of two components. At low oxytocin concentrations only phasic tension waves were produced, whereas at high concentrations an additional tonic component to the mechanical response was seen. The results of the current experiments suggest that these two components are generated by different biochemical mechanisms.

It has been shown that a low concentration of oxytocin (0.2 nM) produced a small but significant increase in $^{45}Ca^{2+}$ influx. No such increase was detected in the presence of higher oxytocin concentrations (2 and 20 nM). These results suggest that the phasic tension development to oxytocin is related to Ca^{2+} influx but the tonic spasm involves another mechanism. It should be noted that Granger *et al.* (1986) demonstrated that concentrations of KCl that produced equivalent tonic mechanical responses to those of oxytocin were associated with a marked increase in $^{45}Ca^{2+}$ influx (up to 65%).

The results obtained with the calcium entry blocker diltiazem also support the view that two biochemical mechanisms underlie the action of oxytocin. Granger *et al.* (1985a,b; 1986) showed that (+)-*cis* diltiazem, and several other calcium entry blockers, were potent inhibitors of phasic tension waves induced by oxytocin (0.2 nM) and inhibited Ca^{2+}-induced spasms and KCl-induced influx of $^{45}Ca^{2+}$ in the rat uterus. In the present study diltiazem exhibited stereospecificity as an inhibitor of the phasic spasms to oxytocin. This

result is in accord with the stereospecificity of diltiazem as an inhibitor of Ca^{2+}-induced spasms of rat uterus (present results), of KCl-induced spasms of guinea-pig ileum and taenia coli (Nagao *et al.*, 1972) and of [^{3}H](+)-*cis* diltiazem binding to a membrane fraction of rat cerebral cortex (Schoemaker & Langer, 1985). These results collectively support the idea that the phasic tension response to oxytocin involves Ca^{2+} influx through voltage-operated Ca^{2+} channels.

cis-Diltiazem was of low potency and did not exhibit stereospecificity as an inhibitor of the tonic component of the spasmogenic response to oxytocin. These observations suggest that diltiazem has actions other than those of calcium entry blockade at high concentrations in accord with earlier observations (Nayler & Horowitz, 1983; Granger *et al.*, 1985a), which explains the ability of diltiazem to inhibit the tonic spasm to oxytocin. These results also support the idea that the tonic spasm does not involve Ca^{2+} influx through voltage-operated Ca^{2+} channels.

Although the spasmogenic responses of the uterus to KCl were qualitatively similar to those of oxytocin, both phasic and tonic spasms to KCl can be entirely explained in terms of Ca^{2+} influx from the extracellular fluid via voltage-dependent Ca^{2+} channels as KCl induced an increase in Ca^{2+} influx and the mechanical response to KCl was completely inhibited by several calcium entry blockers (Granger *et al.*, 1985b, 1986; present study). It is clear that the qualitative nature of the spasmogenic response to an agonist does not necessarily indicate the associated biochemical mechanism which underlies the spasm.

The spasmogenic responses to bradykinin and to oxytocin were qualitatively similar and (+)-*cis* diltiazem selectively inhibited the phasic component of the response to both agonists. Prostaglandin $F_{2\alpha}$ also elicited phasic and tonic spasms of the rat uterus, with the former component selectively inhibited by gallopamil (Reiner & Marshall, 1976) although the tonic component is not as evident as that with oxytocin or bradykinin (Good & Hollingsworth, unpublished observations). It can, therefore, be suggested that the phasic component of the tension response of the uterus to oxytocin, bradykinin and prostaglandin $F_{2\alpha}$ similarly involves Ca^{2+} influx via voltage-dependent Ca^{2+} channels. In addition, all three spasmogens elicit a tonic spasm of the uterus which appears to result from another mechanism(s).

The response to acetylcholine consisted of a single phasic or tonic tension development depending on concentration. The response was less sensitive to inhibition by (+)-*cis* diltiazem than were responses to KCl or phasic responses to oxytocin (Figure 4). Bengtsson *et al.* (1984) also found that gallopamil (1 μM) produced a greater inhibition of spasm to KCl than to acetylcholine. These observations are consistent with the view that acetylcholine produces spasm via a mechanism different from that of KCl or oxytocin (phasic component).

Oxytocin increased spike activity in association with the phasic and tonic spasms. In the presence of a concentration of (+)-*cis* diltiazem which selectively inhibited the phasic but not the tonic spasms, these spikes were also inhibited suggesting that spike activity is necessary for the phasic tension waves.

The tonic spasm to oxytocin (20 nM) was not dependent upon spikes but is likely to be associated with a persistent depolarization (Marshall, 1968; Suzuki & Kuriyama, 1975). Prostaglandin $F_{2\alpha}$ also produces spikes and a persistent depolarization of rat uterus, but while gallopamil abolished the spikes the slow depolarization persisted (Reiner & Marshall, 1976). It is clear from the present studies that the tonic spasm to oxytocin involves neither significant Ca^{2+} influx nor voltage-operated Ca^{2+} channels. This inevitably leads to the concept that the depolarization produced by high concentrations of oxytocin (and probably bradykinin and acetylcholine) does not lead to the opening of voltage-operated Ca^{2+} channels. This surprising situation is similar to the same phenomenon observed in guinea-pig trachealis on exposure to acetylcholine or histamine (Ahmed *et al.*, 1984; 1985). As an increase in cytoplasmic Ca^{2+} concentration appears fundamental to tension development (Bolton, 1979), it might be suggested that the tonic spasm to oxytocin involves the opening of receptor-operated channels and/or increase in phosphatidylinositol turnover (Berridge, 1981), the subsequent release of Ca^{2+} from intracellular stores, decreased Ca^{2+} extrusion or decreased intracellular Ca^{2+} binding. Similar mechanisms presumably underlie the tonic component of the spasm to bradykinin and the spasmogenic response to acetylcholine.

Attempts were made to study further the role of cations in the spasmogenic action of oxytocin, KCl, bradykinin and acetylcholine by comparing the effects of Na^+ and Ca^{2+} removal from the bathing medium on responses to the four agonists. Experiments with a low Na^+ PSS were performed since Reiner & Marshall (1976) had suggested that tonic spasms to prostaglandin $F_{2\alpha}$ were partially dependent on extracellular Na^+. In Ca^{2+}-free PSS there was differential inhibition of the responses to the four agonists. However, their rank order of sensitivity in these experiments did not parallel their relative susceptibilities to inhibition by (+)-*cis* diltiazem. Responses of the four agonists were reduced in a low-Na^+ PSS but there was no differential sensitivity. It therefore seems clear that in uterine smooth muscle reduction of the concentration of a cation in the PSS is not necessarily a useful technique for identifying the role of that ion in a spasmogenic response.

In summary, oxytocin, at low concentrations, produces phasic tension development of the isolated

uterus of the term pregnant rat which can be explained by Ca^{2+} influx via voltage-dependent Ca^{2+} channels. Oxytocin, at high concentrations, in addition produces a tonic spasm resulting from another mechanism which does not appear to involve Ca^{2+} influx. Bradykinin produced qualitatively similar spasmogenic responses to that of oxytocin and appears to act via similar mechanisms. While all the spasmogenic responses to KCl can be explained by Ca^{2+} influx through voltage-dependent Ca^{2+} channels, this mechanism does not appear to be applicable to acetylcholine-induced spasms.

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