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The relaxant action of BRL 34915 in rat uterus

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1 BRL 34915 (0.04–1.3 μM) caused concentration-dependent inhibition of spontaneous phasic spasms of the isolated uterus of the term pregnant rat and this effect was not antagonized by propranolol. Spasms evoked by low concentrations of KCl (< 20 mM) were inhibited by BRL 34915 but those evoked by higher concentrations (> 40 mM) were unaffected.

2 In experiments using extracellular electrical recording, BRL 34915 (10 μM) selectively inhibited oxytocin-induced phasic spasms and the associated spike activity but had little effect on the tonic component of the spasms. BRL 34915, as an inhibitor of phasic spasms to oxytocin (0.2 nM), was antagonized by procaine (0.3 and 1 mM).

3 BRL 34915 (10 μM) did not inhibit Ca^{2+}-induced spasm of saponin-skinned thin myometrial strips.

4 Intracellular microelectrode recording from myometrial strips showed that BRL 34915 (10 μM) inhibited action potentials and phasic spasms in the presence of oxytocin (0.2 nM) and produced a hyperpolarization of 5 mV.

5 In single myometrial cells under current or voltage clamp, BRL 34915 (10 μM) had no effect on action potentials and inward current in Ca^{2+}- or Ba^{2+}-containing media in the presence of tetraethylammonium, 4-aminopyridine and caesium chloride. In the absence of these K^+-channel inhibitors, BRL 34915 had no effect on resting membrane potential, membrane resistance, action potentials, inward current or outward current.

6 BRL 34915 (1 or 10 μM) had no effect on ^{86}Rb efflux from myometrial strips. ^{86}Rb efflux was increased by oxytocin (0.2 and 20 nM).

7 The relaxant profile of BRL 34915 in the rat uterus is similar to that described for other smooth muscles where an action to open membrane K^+-channels has been proposed. BRL 34915 inhibited spike production but produced only a small hyperpolarization without a detectable increase in ^{86}Rb efflux. Membrane resistance and transmembrane currents were unaffected. These results suggest that in the uterus the effects of BRL 34915 may be restricted to K^+-channels involved in the production of pacemaker activity.

Introduction

BRL 34915 can relax rat isolated portal vein and aorta (Hamilton *et al.*, 1986; Weir & Weston, 1986b; Southerton *et al.*, 1987), guinea-pig taenia coli (Weir & Weston, 1986a), guinea-pig trachealis (Allen *et al.*, 1986) and rabbit mesenteric artery (Clapham & Wilson, 1986). It has been suggested that these inhibitory effects are achieved by the opening of K^+-channels so that the membrane potential of smooth muscle cells is held at or close to the potassium equilibrium potential (E_K) (Allen *et al.*, 1986; Hamilton *et al.*, 1986; Weir & Weston, 1986a,b). Support for this idea comes from the ability of BRL 34915 to inhibit spasm produced by low (< 40 mM) but not high (> 40 mM) concentrations of KCl. Also inhibition of spontaneous mechanical activity in the above tissues is associated both with hyperpolarization to a potential close to E_K and with increased efflux of ^{86}Rb.

In double sucrose gap experiments with the myometrium of the pregnant rat, two types of outward K^+ current have been described (Mironneau & Savineau, 1980; Mironneau *et al.*, 1981). One of these is rapidly activated and inactivated and can be inhibited by tetraethylammonium (TEA) and 4-aminopyridine. The other current is more slowly activated and

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inactivated and can be inhibited by TEA only.

The objective of this study was to determine the effects of BRL 34915 on the electrical and mechanical activity of the rat uterus. It was hoped that such observations would provide further information about the mechanism of action of BRL 34915, particularly with regard to its K^+-channel opening properties, and more insight into its tissue selectivity. Preliminary results have been communicated (Edwards *et al.*, 1987).

Methods

Uteri were obtained from day-22 pregnant Sprague-Dawley rats (250–350 g) supplied by the Manchester University Animal Unit or day 18–19 pregnant Wistar rats (saponin-skinned preparations, single myometrial cells). Uterine horns were freed of foetuses and placentae and placed in a physiological salt solution (PSS) at room temperature.

Tissue bath experiments

Mechanical responses of longitudinal strips of uterus were recorded isometrically at 37°C and measured as the integrated tension (Granger *et al.*, 1985; 1986). The effect of BRL 34915 against spontaneous phasic tension development was assessed by its cumulative addition at 10 min intervals and expressing the integral in the last 5 min of each interval as a % of the control integral. Similar experiments were performed using tissues whose mechanical activity was enhanced by the presence of oxytocin (0.2 nM) in the PSS. In these tissues, cumulative concentration-effect curves to BRL 34915 were constructed both before and after tissue equilibration (30 min) with procaine (0.3 and 1 mM). In other experiments the uterus was exposed to KCl or oxytocin and their respective concentrations increased in a cumulative manner every 10 min. Concentration-effect curves were constructed before and 10 min after incubation of tissues with and in the continued presence of increasing concentrations of BRL 34915.

Saponin-skinned preparation

Saponin-skinning was performed by the method of Savineau *et al.* (1986). Thin strips of longitudinal muscle (50–80 μm in diameter, 2–3 mm in length), free of both endometrium and circular muscle, were mounted for recording of isometric tension using an Akers AE 801 transducer (Aksjeselskapet Micro-Electronikk, Norway). The tissue was continuously superfused with a PSS of the following composition (mM): KCl 5.6, NaCl 130, $CaCl_2$ 2.1, $MgCl_2$ 0.24, Tris 8.3, glucose 11, pH 7.4, bubbled with 95% O_2, 5% CO_2 at 25°C. Spasms were obtained to K^+ (10 and 40 mM) before and after 5 min incubation with BRL 34915 (10 μM). The tissue was then superfused with saponin (50 μg ml^{-1}) for 20 min in a relaxing solution which had the following composition (mM): potassium methanesulphonate 135, magnesium methanesulphonate 5, disodium adenosine triphosphate 5, ethylene-glycol-bis (β-aminoethylether)-N,N′-tetracetic acid (EGTA) 4, tris (hydroxymethyl) aminomethane 20, maleic acid 20, pH adjusted to 6.8 with KOH at 25°C. Subsequently the tissue was bathed in the same medium without saponin. Ca^{2+} spasms were then obtained by addition of appropriate Ca methanesulphonate concentrations to the relaxing solution. The free Ca^{2+} concentrations were calculated according to the method of Fabiato & Fabiato (1979). BRL 34915 (10 μM) was added when the maximum tension to added Ca^{2+} had developed.

Extracellular electrical recording

Simultaneous measurement of tension development and extracellular electrical activity was performed as described by Granger *et al.* (1986) and Edwards *et al.* (1986). Longitudinal strips of uterus were superfused with normal PSS and oxytocin (0.2, 2 and 20 nM) was added cumulatively at 10 min intervals. Strips were then equilibrated (10 min) with normal PSS (controls) or PSS containing BRL 34915 (10 μM) before the oxytocin challenge was repeated.

Intracellular microelectrode recording from tissue strips

The intracellular microelectrode technique as described by Small & Weston (1980) for flat tissues was used. Longitudinal strips of uterus were mounted with their serous surface uppermost such that longitudinal mechanical activity and electrical activity could be recorded simultaneously. Oxytocin (0.2 nM)-enhanced activity was recorded from an impaled myometrial cell for 2 min before and in the presence of BRL 34915 (10 μM) for 8 min.

Single myometrial cells

Single myometrial cells from 18–19 day pregnant rats were prepared in short-term primary culture by the technique of Amédée *et al.* (1986) and Mollard *et al.* (1986). In outline, this involved dissociation of cells using five successive incubations with collagenase (0.1%) and plating onto collagen-coated coverslips. Cells were used between 12 h and 50 h after dissociation. Electrophysiological recordings were made at 35°C using intracellular microelectrodes by the technique of Amédée *et al.* (1986) and Mollard *et al.* (1986).

In brief, membrane potentials were recorded with

conventional microelectrodes filled with 3 M KCl. The potential difference between the intracellular microelectrode and a bath reference electrode was measured with a Dagan 8100 system (Dagan, Minneapolis, U.S.A.), using the switched mode (switch frequency between 3 and 15 kHz). Currents were injected into the cell through the recording microelectrode.

Microelectrodes with resistances ranging from 30 to 50 MΩ were pulled from borosilicate capillary tubes (Clark Electromedical Instruments, Reading). To adjust the leakage current flowing through the recording circuit, a 300 MΩ resistor (1%, Victoreen), in parallel with a 100 pF capacitor, was connected between the input stage and the ground. The recorded potential was then adjusted to less than ± 1 mV, indicating leakage current of less than ± 4 pA.

Action potentials and currents induced by intracellular stimulation were produced with cells incubated in a PSS containing $CaCl_2$ (10 mM) or $BaCl_2$ (10 mM). Maximum inhibition of outward K^+ currents was obtained by tetraethylammonium (TEA, 10 mM) and 4-aminopyridine (10 mM) in the PSS and by the intracellular diffusion of $CsCl_2$ (4 M) from the microelectrodes. Action potentials (elicited by injections of a depolarizing current pulse of ≃100 pA, 30–50 ms) in $CaCl_2$- or $BaCl_2$-containing media or inward currents in $BaCl_2$-containing medium (triggered by depolarizing pulses (200–300 ms) from a holding potential of −60 mV to −10 mV) were recorded in control solution and at 1 min intervals after the commencement of perfusion with the same medium plus BRL 34915 (10 μM).

The effect of BRL 34915 on membrane properties of cells incubated in PSS without TEA or 4-aminopyridine and containing $CaCl_2$ (3.6 mM) was also measured. The microelectrodes contained KCl (3 M). Resting membrane potential, action potentials and membrane resistance (to hyperpolarizing current pulses of 35 pA, 200 ms) were measured in control solution and at 1 min intervals after commencement of perfusion with BRL 34915 (10 μM). Transient (peak) and steady-state (sustained) outward currents were measured to a range of depolarizing voltage steps from a holding potential of −60 mV to between −50 mV and +10 mV for 200–300 ms in a PSS containing 10 mM Ca^{2+}. These currents were obtained in control PSS and 2 min after the commencement of perfusion with BRL 34915 (10 μM).

The other components of the PSS were (mM): Na^+ 130, K^+ 5.6, Mg^{2+} 0.24, Cl^- 135.8, glucose 11. The PSS was buffered with tris (hydroxymethyl) aminomethane-HCl (8.3 mM) at pH 7.4.

^{86}Rb efflux

In these experiments ^{86}Rb was used as a K^+ marker and the technique of Hamilton *et al.* (1986) was broadly followed. Myometrial strips were removed from 4 day-22 pregnant rats on each of 2 days and assigned to the following experimental groups: day 1 – PSS, PSS + BRL 34915 vehicle, BRL 34915 (10 μM); day 2 – PSS, BRL 34915 (1 μM), BRL 34915 (10 μM), oxytocin (0.2 or 20 nM).

Tissues were attached to a gassing manifold and after a 10 min equilibration period at 37°C were loaded with ^{86}RbCl, 5 μCi ml^{-1} (185 MBq l^{-1}), for 90 min. ^{86}Rb was then allowed to efflux from the tissue into 5 ml aliquots of PSS using 2 min collection periods. After the initial efflux into PSS for 8 such periods (16 min into the efflux), the tissues were exposed to PSS alone or PSS plus drug or PSS plus vehicle as indicated above for the next 4 collection periods. For the remaining 3 periods, the tubes contained PSS alone.

At the end of the efflux period, 1 ml aliquots of efflux media were added to 9 ml OptiPhase Safe (LKB) scintillation mixture and counted for radioactivity. Each strip was blotted, weighed (range 3–9 mg) and digested in 0.5 ml Soluene 350 (Packard). To the resulting solution, 0.5 ml 0.5 N hydrochloric acid and 9 ml OptiPhase Safe was added and the mixture counted for radioactivity. The efflux data were measured in terms of rate coefficient (fractional loss of ^{86}Rb from the tissue for a 1 min period, expressed as a %). Efflux was then standardized for each myometrial strip by determining the mean rate coefficient over the 10–16 min periods and all data for that strip were expressed as a % of this mean.

In tissue bath studies, KCl produced concentration-related tension changes in myometrial strips similar to those seen in whole uterus. BRL 34915 (1 and 10 μM) produced antagonism of spasms to 10 and 20 mM KCl but not to 40 and 80 mM KCl (data not shown).

Drugs and solutions/statistical analysis of data

The following chemicals were used: (±)-BRL 34915 ((±)-6-cyano-3,4-dihydro-2, 2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol, Beecham), oxytocin (grade X, Sigma), salbutamol sulphate (Glaxo), (±)-propranolol hydrochloride (I.C.I.), saponin (I.C.N., Cleveland, U.S.A.) and EGTA (Sigma, St Louis, U.S.A.) ^{86}RbCl (8 mCi mg^{-1}) was obtained as an aqueous solution from Amersham International. The stock solution of BRL 34915 (10 mM) was prepared in 70% v/v ethanol:distilled water, salbutamol was prepared in 0.1 N hydrochloric acid and other drugs in twice distilled water. The PSS (for tissue bath, extracellular and intracellular recording and ^{86}Rb efflux experiments) had the following composition (mM): Na^+ 143.0, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.55, H_2PO_4 1.2, SO_4^{2-} 1.2, Cl^- 128.0, HCO_3 25.0, glucose 11.0 and was bubbled with 95% O_2 and 5%

CO_2. Where KCl was used as the spasmogen the stated concentration excludes the KCl present in the PSS.

The significance of differences between means was assessed by two-tailed un-paired Students t test or by analysis of variance and the least significant difference test.

Results

Tissue bath and extracellular electrical recording

BRL 34915 (40 nM-1.3 μM) produced concentration-related inhibition of spontaneous phasic spasms with an IC_{50} of 6.70 ± 0.24 ($-\log$ M, mean ± s.e.mean, $n = 8$). The onset of the effect occurred within 1 min and was maximal by 5 min. Salbutamol (0.1 nM–12.5 nM) also suppressed spontaneous mechanical activity. Propranolol (0.1 μM) did not antagonize BRL 34915 but produced a 15 fold antagonism of salbutamol. In control experiments (Figure 1a) the shape and position of the log concentration-effect curve for KCl did not change with time. In test tissues BRL 34915 (1 and 10 μM) significantly reduced spasms evoked by KCl (Figure 1b). Responses to 10 mM KCl were abolished by BRL 34915, those to 20 mM KCl were reduced but the drug had no effect on the responses to 40 and 80 mM KCl.

Oxytocin (0.2 nM) produces an increase in the amplitude and frequency of phasic spasms while an additional tonic component is seen with higher concentrations (2 and 20 nM) (Edwards *et al.*, 1986). In control experiments the log concentration-effect curve of oxytocin was slightly but progressively depressed when repeated in PSS (Figure 2a). In test tissues BRL 34915 (0.1, 1 and 10 μM) produced a significant concentration-dependent reduction of spasms to oxytocin with greater inhibition of responses to low than to high concentrations of oxytocin due to selective inhibition of the phasic component (Figure 2b). These observations were extended using the extracellular electrical recording technique. As shown by Edwards *et al.* (1986) and in Figure 3, phasic spasms to oxytocin (0.2 nM) were temporally associated with spike bursts while continuous electrical spiking was seen at least initially during tonic spasms to oxytocin (20 nM). In control experiments such responses to oxytocin were reproducible when a second concentration-effect curve was constructed. In test tissues BRL 34915 (10 μM) selectively inhibited the phasic component of the spasm and the associated spikes at any concentration of oxytocin. It had much less effect on the tonic component of the spasm which, in the presence of BRL 34915, was not associated with spikes. Occasional spike discharges were observed in response to 20 nM oxytocin in the presence of BRL 34915 (10 μM) but these were always associated

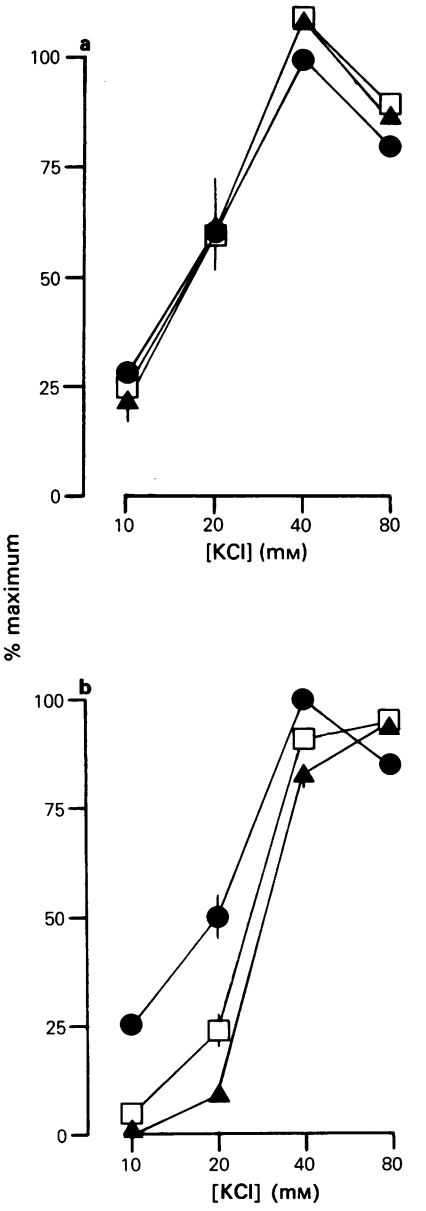

Figure 1 Effect of BRL 34915 on mechanical responses to KCl in the uterus of the term pregnant rat. Ordinate scales: responses expressed as a % of the initial response to KCl (40 mM). Abscissa scales: concentrations of KCl on a log scale. (a) Time-matched concurrent controls; (b) initial responses (●) and responses after 10 min incubation with BRL 34915 1 μM (□), 10 μM (▲). The points are the means and the vertical lines show the s.e.means ($n = 5$–6).

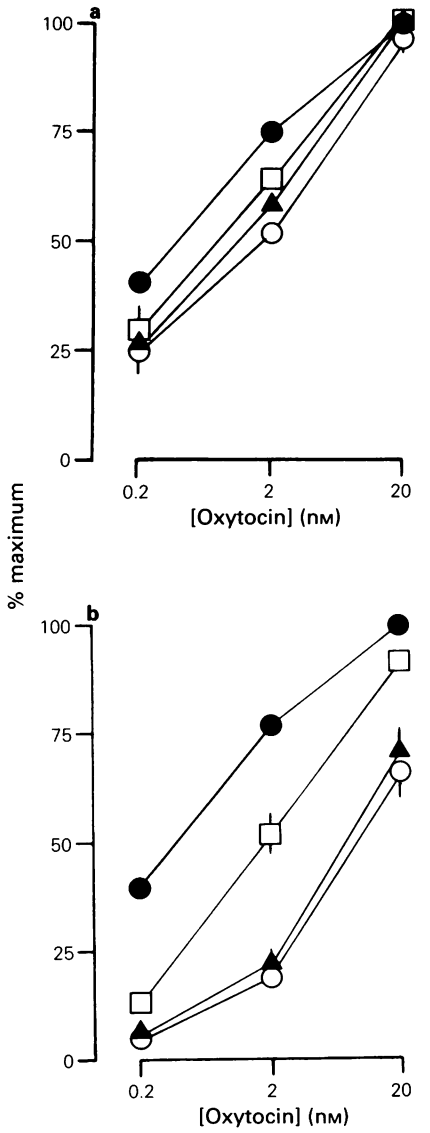

Figure 2 Effect of BRL 34915 on mechanical responses to oxytocin in the uterus of the term pregnant rat. Ordinate scales: responses expressed as a % of the initial response to oxytocin (20 nM) measured as integral ($g\,s^{-1}$). Abscissa scales: concentrations of oxytocin on a log scale. (a) Time-matched concurrent controls; (b) initial responses (●) and responses after 10 min incubation with BRL 34915 0.1 μM (□), 1 μM (▲), 10 μM (○). The points are the means and the vertical lines show the s.e.means ($n = 7-8$).

with phasic spasms. BRL 34915, as an inhibitor of phasic spasms to oxytocin (0.2 nM), was antagonized by procaine. Procaine (0.3 and 1 mM) produced log concentration-ratios for BRL 34915 of 0.33 ± 0.17 and 1.55 ± 0.31, respectively ($n = 6$), which were significantly ($P < 0.05$) different from log concentration-ratios in time-matched control tissues. There was no change in the potency of BRL 34915 in control tissues.

Saponin-skinned preparations

In the intact thin myometrial strip, BRL 34915 (10 μM) completely blocked the spasm to K^+ 10 mM but had no effect on the spasm to K^+ 40 mM (Figure 4a). After saponin-skinning, added Ca^{2+} (from pCa > 8 to pCa = 5.5) produced concentration-dependent spasms which reached a peak tension by 1.5 min and were maintained for at least 10 min. BRL 34915 (10 μM), or equivalent volume of solvent, was added to the perfusing medium when the tension to Ca^{2+} had reached a maximum. BRL 34915 did not modify these spasms (Figure 4b).

Intracellular microelectrode recording with tissue strips

The electrical activity in the control situation was similar to that described by Casteels & Kuriyama (1965). The resting membrane potential was -55.1 ± 2.2 mV ($n = 4$). Oxytocin (0.2 nM)-enhanced phasic spasms were associated with bursts of spike potentials (Figure 5). Superfusion of these tissues with PSS containing BRL 34915 (10 μM) produced a cessation of these potentials and spasms within 2 min (Figure 5). Intracellular recordings were continued for up to 8 min after initial drug contact and the maximum hyperpolarization observed was 5.1 ± 1.3 mV ($n = 4$) to a membrane potential of -60.2 ± 1.5 mV.

Single myometrial cells

The objective of the first series of experiments was to examine the effects of BRL 34915 against Ca^{2+} and Ba^{2+} action potentials and Ba^{2+} inward currents. Electrically-induced action potentials and inward currents with cells bathed in a solution containing Ca^{2+}, TEA and 4-aminopyridine and using microelectrodes containing $CsCl_2$ were of the form described previously (Mollard *et al.*, 1986). The action potentials in Ca^{2+}-containing PSS had amplitudes of 102.3 ± 4.8 mV and a duration at 50% repolarization of 42.5 ± 10.1 ms (Table 1). Action potentials in Ba^{2+}-containing PSS were of the same amplitude but of longer duration. There was no change in the magnitude or shape of action potentials or inward currents when measured at 1 min intervals up to 5 min after the commencement of perfusion with BRL 34915 (10 μM) (Table 1).

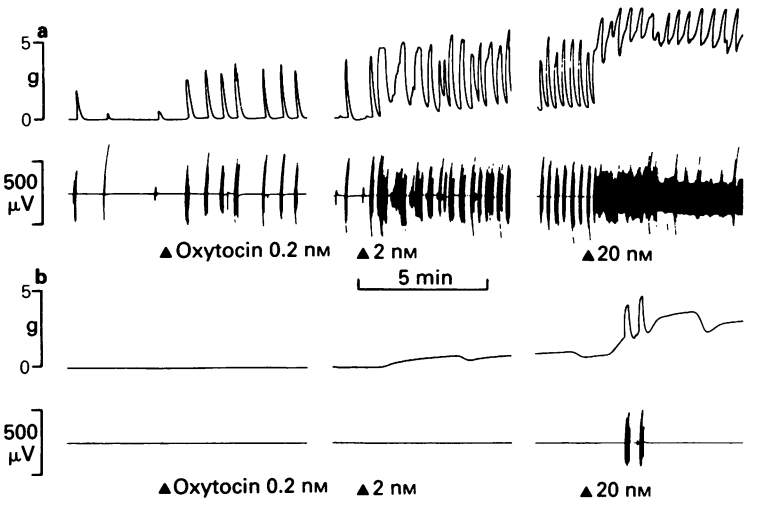

Figure 3 Effect of BRL 34915 on oxytocin-driven mechanical activity (upper traces) and extracellularly-recorded electrical activity (lower traces) of uterus from the term pregnant rat. Recordings are from the same tissue (a) before and (b) 10 min after the commencement of superfusion with PSS containing 10 μM BRL 34915.

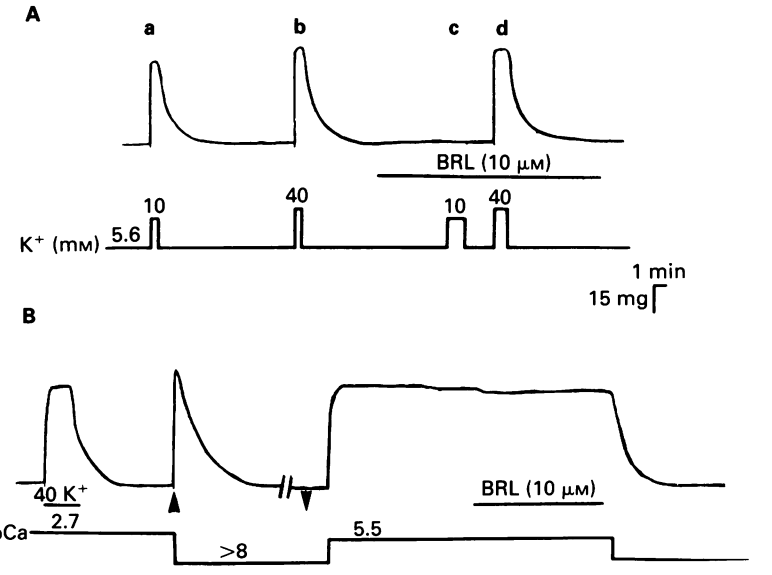

Figure 4 The lack of effect of BRL 34915 on the maintenance of spasm evoked by Ca^{2+} in saponin-skinned myometrial strips. (A) Control experiment showing the effect of BRL 34915 (10 μM) on potassium (K^+)-induced spasms in thin bundles. Spasms were evoked by 10 or 40 mM K^+ before (a,b) and 5 min after (c,d) addition of BRL 34915 (10 μM). (B) Skinned myometrial strip. After recording a control response to 40 mM K^+ in the intact strip, the relaxing solution containing 50 μg ml^{-1} saponin was introduced (▲) for 20 min. Subsequently, saponin was washed out (▼) and after about 2 min a contracting solution (pCa = 5.5) was added. A near maximum contraction developed, the amplitude of which was not modified by the addition of BRL 34915 (10 μM) for 9 min. Experiments were performed at 25°C.

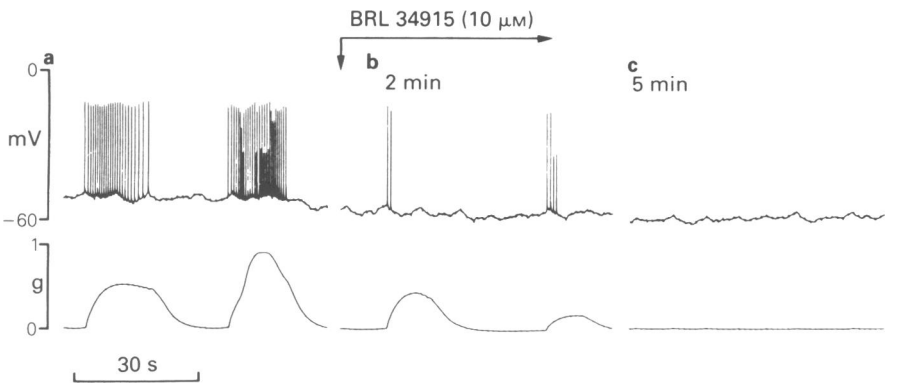

Figure 5 The effect of BRL 34915 (10 μM) on the electrical (upper trace) and simultaneously recorded mechanical (lower trace) activity of the uterus of the term pregnant rat. The electrical trace is part of a continuous impalement of a single cell. Activity is shown (a) before, (b) 2 min and (c) 5 min after the commencement of perfusion with BRL 34915 (10 μM). The tissue was perfused throughout with oxytocin (0.2 nM). Note the ability of BRL 34915 to produce relaxation, suppress spikes and cause a small hyperpolarization.

Table 1 Lack of effect of 5 min incubation with BRL 34915 (10 μM) on action potentials in Ca^{2+}- and Ba^{2+}-containing solutions or transmembrane inward current in Ba^{2+}-containing media in single myometrial cells

	Control	*BRL 34915 (10 μM)*
Ca^{2+}		
Action potential		
Amplitude (mV)	102.3 ± 4.8	98.0 ± 6.3 (4)
Duration (ms)	42.5 ± 10.1	56.3 ± 8.0 (4)
Ba^{2+}		
Action potential		
Amplitude (mV)	95.0 ± 5.9	89.0 ± 7.5 (3)
Duration (ms)	235 ± 100	161 ± 45 (3)
Inward current		
Amplitude (pA)	458 ± 30	426 ± 65 (3)

Duration was defined as the time between the peak of the action potential and 50% repolarization. The medium contained $CaCl_2$ (10 mM) or $BaCl_2$ (10 mM) plus tetraethylammonium (10 mM), 4-aminopyridine (10 mM) and the microelectrodes $CsCl_2$ (4 M). Values are means ± s.e.mean; number of cells in parentheses.

The purpose of the second series of experiments was to study the effects of BRL 34915 on membrane properties in the absence of the K^+-channel blockers, TEA and 4-aminopyridine. The resting membrane potential (−52.4 ± 4.7 mV, $n = 6$), input resistance (847 ± 192 MΩ, $n = 6$) and amplitude of action potentials (67.8 ± 4.2 mV, $n = 6$) in Ca^{2+}-containing PSS in the absence of BRL 34915 were similar to those described previously (Mollard *et al.*, 1986). Perfusion with BRL 34915 (10 μM) for 5 min did not change these parameters. Two further cells were perfused with a PSS containing a reduced K^+ concentration (2 mM) for 2 min before perfusion with PSS containing K^+ (2 mM) plus BRL 34915 (10 μM) for 5 min in the hope of augmenting any effects of BRL 34915. Again BRL 34915 had no effect on resting membrane potential or action potentials.

Voltage clamp experiments were performed to examine more directly the effect of BRL 34915 on outward K^+ currents. Depolarizing voltage steps performed in control solution (10 mM Ca^{2+}) produced an initial inward current followed by an outward current. The latter current usually exhibited an early peak followed by a smaller sustained value (Figure 6Aa). The relationships between the voltage step and both the peak outward current and the outward current at the end of the depolarizing step were as shown in Figure 6Ba. The shape of the outward current-time curve and the characteristics of the two outward current:voltage relationships were unchanged when repeated from 2 min after the commencement of perfusion with BRL 34915 (10 μM) (Figure 6Ab, Bb).

^{86}Rb efflux experiments

There was an initial rapid decline in the rate coefficient of ^{86}Rb efflux in the first three 2 min efflux periods (data not shown). Subsequently, in control tissues, the rate coefficient of ^{86}Rb efflux did not change with time (Figure 7). Oxytocin (0.2 and 20 nM, $n = 2$ and 1 respectively) significantly increased the efflux of ^{86}Rb

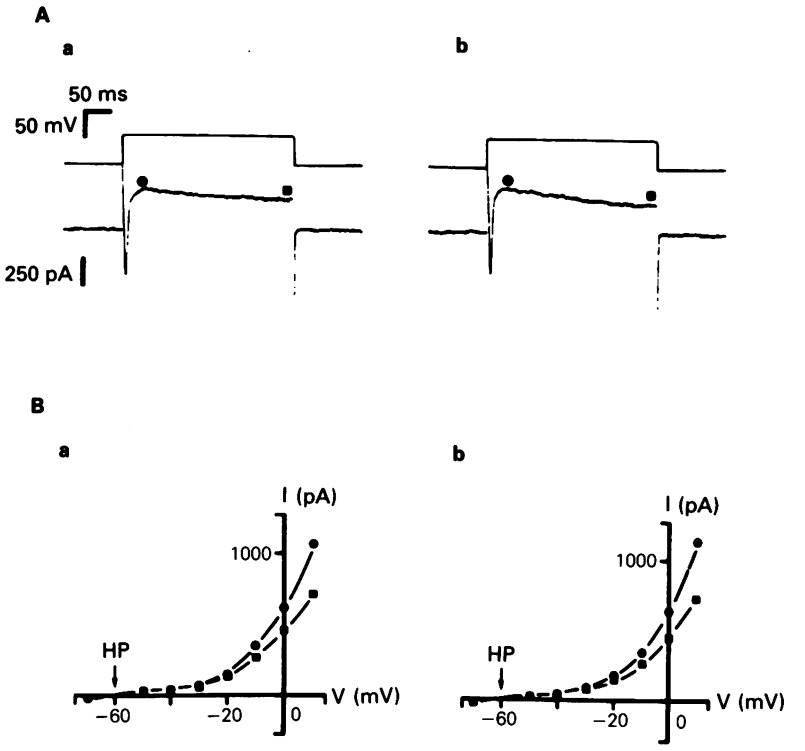

Figure 6 Voltage clamp experiments on a single myometrial cell. (A) Inward and outward currents produced by a single depolarizing voltage step from a holding potential (HP) of −60 mV to −10 mV in an isolated myometrial cell (a) in control PSS and (b) 2 min after commencement of incubation in BRL 34915 (10 μM). (B) Current (I)-voltage (V) relationships for the same cell (a) in control PSS and (b) in the presence of BRL 34915 (10 μM). Values for current were taken at the peak outward current (●) and at the end of the depolarizing step (■). These results were typical of 3 cells. The PSS contained Ca^{2+} (10 mM) and the microelectrode KCl (3 M).

($P < 0.01$; analysis of variance). BRL 34915 (1 or 10 μM; $n = 4$ and 7, respectively) did not change the ^{86}Rb efflux rate coefficient.

Discussion

The effects of BRL 34915 on mechanical activity in the rat uterus are similar to those described in other smooth muscles. The compound is a potent inhibitor of spontaneous tone in the guinea-pig trachealis (Allen *et al.*, 1986), guinea-pig taenia caeci (Weir & Weston, 1986a) and of spontaneous phasic spasms in the rat portal vein (Hamilton *et al.*, 1986) and rat uterus (present study). A characteristic observation is that BRL 34915 inhibits spasms to low but not high concentrations of KCl in these tissues. The potency of BRL 34915 as an inhibitor of spontaneous and KCl-induced spasms in the uterus is similar to that observed in vascular, tracheal and gastrointestinal tract tissues. Therefore, BRL 34915 has not to date exhibited significant selectivity for different types of isolated smooth muscles. This observation, plus the similarity of pharmacodynamic actions of these tissues, would suggest a common mechanism of action of BRL 34915 in these preparations.

It is clear that the mechanism of relaxation produced by BRL 34915 in the uterus is very different from that produced by the calcium entry blockers. Several calcium entry blockers are, like BRL 34915, able to inhibit spontaneous phasic spasms of the uterus (Granger *et al.*, 1985). However, these compounds are also able to inhibit spasms to all concentrations of KCl (Granger *et al.*, 1986), an action quite distinct from that of BRL 34915. Support for the idea that action potentials and inward current in single myometrial cells is carried by divalent ions comes from their sensitivity to Mn^{2+} ions, Co^{2+} ions, gallopamil and changes in extracellular Ca^{2+} ion concentration but insensitivity to tetrodotoxin and changes in extracellular Na^{+} ion concentration (Mollard *et al.*, 1986). We have now shown directly that BRL 34915

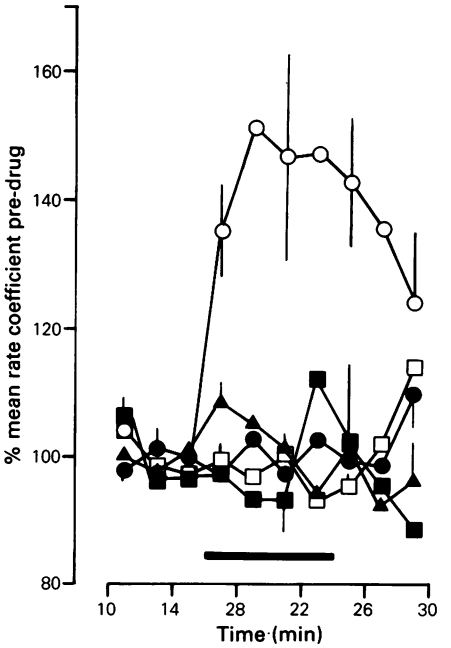

Figure 7 Effects of BRL 34915 1 μM (■), BRL 34915 10 μM (▲), oxytocin 0.2 and 20 nM pooled (○), 0.07% v/v ethanol (□) and PSS alone (●) on the efflux of ^{86}Rb from rat myometrial strips. Ordinate scale: ^{86}Rb efflux rate coefficient expressed as % of the mean rate coefficient derived from the 3 periods preceding exposure to drug or solvent. Abscissa scale: time (min) after start of the efflux period. Tissues were exposed to the drugs or vehicle between the 16th and 24th min of the efflux period as indicated by the bar. Each point is the mean derived from 3–7 tissues; vertical lines show representative s.e.means.

does not inhibit these Ca^{2+} or Ba^{2+} action potentials or Ba^{2+} inward current in the presence of K^+ conductance inhibitors. The relaxation to BRL 34915 is not mediated via β-adrenoceptors as the effect was not antagonized by propranolol.

Allen *et al.* (1986) have shown that BRL 34915 did not reduce spasm induced by Ca^{2+} in Triton-X100 skinned trachealis. They concluded that BRL 34915 does not directly reduce the sensitivity of the intracellular contractile machinery to Ca^{2+}. We have now shown in the saponin-skinned myometrium that BRL 34915 did not affect Ca^{2+} spasms. It is clear that BRL 34915 does not directly affect the uterine contractile apparatus. The observation that BRL 34915 selectively inhibits the voltage-dependent (phasic) component of the action of oxytocin in rat uterus further supports the idea of a site of action for BRL 34915 associated with the plasma membrane.

Substantial evidence has been provided that the mechanism of the relaxant action of BRL 34915 in tissues other than the uterus involves the opening of K^+-channels. BRL 34915 is able to produce a concentration-dependent hyperpolarization of rat portal vein (Hamilton *et al.*, 1986; Weir & Weston, 1986b), rat aorta (Southerton *et al.*, 1987) and guinea-pig trachealis (Allen *et al.*, 1986). The maximum membrane potentials obtained in these tissues in the presence of BRL 34915 were close to the calculated E_K. However, in the rat uterus, which was as sensitive to the relaxant actions of BRL 349165 as portal vein and trachealis, the rapid and complete abolition of phasic spasms by BRL 34915 (10 μM) was associated with a hyperpolarization of only 5 mV which raised the membrane potential to −60 mV. Based on measurements of ionic content or ionic fluxes, the calculated E_K in the rat uterus is between −77 mV and −90 mV (Casteels & Kuriyama, 1965; Hamon *et al.*, 1976). Mironneau & Savineau (1980) and Mironneau *et al.* (1981) have described two components (fast and slow) of the outward (K^+) current in rat myometrium using the double sucrose gap technique. The reversal potential for the fast outward current was −80 mV (Mironneau *et al.*, 1981). The slow current appears to have a reversal potential of −65 mV (Mironneau *et al.*, 1981; unpublished observations). One explanation of the limited hyperpolarization with BRL 34915 is, therefore, that the compound acts to open only the slow K^+ channel and hence can only elevate the membrane potential to −65 mV.

^{86}Rb has been used as a marker for the movement of K^+ in smooth muscle (Bolton & Clapp, 1984; Hamilton *et al.*, 1986). The relaxant effects of BRL 34915 are associated with the increased efflux of ^{86}Rb from the rat portal vein and aorta (Hamilton *et al.*, 1986; Weir & Weston, 1986b; Southerton *et al.*, 1987), rabbit mesenteric artery (Coldwell & Howlett, 1986), guinea-pig taenia coli (Weir & Weston, 1986a) and guinea-pig trachealis (Allen *et al.*, 1986). ^{86}Rb has now been shown to exchange into and efflux from rat myometrium. However, BRL 34915 did not alter ^{86}Rb efflux in rat myometrium at a concentration which was sufficient to inhibit spontaneous and KCl-induced spasms. It could be argued that K^+-channels opened by BRL 34915 do not easily allow the exchange of ^{86}Rb in rat myometrium, as discussed for rat aorta (Weir & Weston, 1986b). Experiments are in progress to test this possibility. Alternatively, the lack of effect of BRL 34915 on ^{86}Rb efflux may be the net consequence of a reduced ^{86}Rb efflux through repolarizing K^+-channels, due to inhibition of spontaneous phasic spasms, and increased efflux, due to the opening of other K^+-channels. Spasmogen action in other smooth muscles is associated with an increased ^{86}Rb efflux (Bolton & Clapp, 1984), presumably due to enhanced opening of Ca^{2+}-dependent K^+-channels. Such ^{86}Rb efflux was observed with oxytocin in the present study and served to demonstrate that

increased ^{86}Rb exchange could be detected with the methodology used in these experiments. A third possibility is that BRL 34915 does not increase K^+ efflux in rat myometrium sufficiently to be detected by the ^{86}Rb technique.

In uterine strips BRL 34915 clearly lacks the effects on membrane potential and ^{86}Rb efflux that have been observed in other smooth muscles. The electrophysiological observations were, therefore, extended to voltage- and current-clamped myometrial cells which have been shown to retain many of the mechanical and electrical properties of tissue strips (Amédée *et al.*, 1986; Mollard *et al.*, 1986). The lack of effect of BRL 34915 on membrane potential, membrane resistance or K^+ outward currents is consistent with the failure to observe marked hyperpolarization or increased ^{86}Rb efflux. This might indicate that K^+ channel opening in the uterus, and perhaps in other tissues, is not causally related to the mechanical inhibition. However, the K^+-channel blocking agent procaine (Yamanaka *et al.*, 1985) reduced the relaxant and hyperpolarizing actions of BRL 34915 in guinea-pig trachealis and its relaxant effect in rat uterus (Allen *et al.*, 1986; present study). Although BRL 34915 had no effect on action potentials induced by depolarizing currents in single cells, paradoxically inhibition of spontaneous action potentials was observed in uterine strips. These observations might suggest that the inhibitory effects of BRL 34915 in uterus involves an action on pacemaker currents. Experiments are planned to test this hypothesis.

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