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RELAXIN INHIBITS THE PLATEAU COMPONENT OF THE ACTION POTENTIAL IN THE CIRCULAR MYOMETRIUM OF THE RAT

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SUMMARY

1. The effects of relaxin on contractility and membrane potential of the longitudinal and circular smooth muscle layers of the uterus have been studied *in vitro* using oestrogen-treated, non-pregnant rats and pregnant rats.

2. Relaxin decreased the amplitude of contractions induced by electrical stimulation of longitudinal myometrium by decreasing the duration of the bursts of action potentials. This effect was transient and tachyphylaxis always developed and was observed following injection of steroids and up to day 17 of pregnancy. There was no inhibition of tissues from rats from day 18 of pregnancy to term. The peptide had no effect on resting membrane potential, space constant or time constant.

3. Action potentials recorded from circular myometrium of non-pregnant rats pre-treated with oestrogen consisted of an initial spike or short burst of spikes followed by a prolonged plateau of depolarization. Spontaneous action potentials and associated contractions were abolished within 2 min of exposure to relaxin (10^{-8} g/ml) while contractions of much smaller amplitude could be evoked with depolarizing current pulses. This effect was associated with depression of the plateau component of the action potential whereas the spike component was left intact. Relaxin had no effect on passive membrane properties.

4. The action potentials of circular myometrium of rats up to day 21 of pregnancy were qualitatively similar to those recorded in the same muscle layer from oestrogen-treated, non-pregnant rats and the plateau component was also blocked by relaxin in these tissues. Bursts of spikes were observed in circular strips 24–36 h before parturition, and the effect of the peptide on these was a transient inhibition similar to that observed in longitudinal myometrium.

5. Oxytocin increased the amplitude of the spike and the amplitude and duration of the plateau. Relaxin abolished the plateau in the presence of 10^{-11} and 10^{-10} M-oxytocin but was ineffective when the concentration of the spasmogen was increased further. Prostaglandin $F_{2\alpha}$ increased the amplitude and duration of the plateau. Relaxin abolished the responses to 10^{-10} and 10^{-9} M-prostaglandin $F_{2\alpha}$.

6. The results of this study demonstrate that relaxin specifically inhibits contractions in the circular layer of the myometrium by abolishing the plateau component

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of the action potential. This action appears to be different from that of other smooth muscle relaxants tested in these experiments (isoprenaline, papaverine and verapamil). All of these abolished simultaneously both the spike and plateau components of the action potential.

INTRODUCTION

It has been shown that exogenous relaxin inhibits uterine contractions in many species (rat: Sawyer, Frieden & Martin, 1953; Bradshaw, Downing, Moffatt, Hinton & Porter, 1981; mouse: Wiqvist, 1959; hamster: Khaligh, 1968; human: Szlachter, O'Byrne, Goldsmith, Steinetz & Weiss, 1980; guinea-pig: Porter, 1972; sheep: Porter, Lye, Bradshaw & Kendall, 1981). Since the first report describing the amino acid sequence of purified porcine relaxin (James, Niall, Kwok & Bryant-Greenwood, 1977) and its tertiary structure (Isaacs, James, Niall, Bryant-Greenwood, Dodson, Evans & North, 1978) attention has turned towards gene isolation, synthesis and sequencing of human relaxin by use of an homologous porcine relaxin cDNA probe (Hudson, Haley, John, Cronk, Crawford, Haralambidis, Tregear, Shine & Niall, 1983). In view of the progress that has been made in characterizing the structure of this peptide it seemed appropriate to undertake further studies which might help to explain its physiological role during pregnancy and parturition.

This report describes observations made during the simultaneous recording of membrane potential and mechanical activity, in an attempt to elucidate the mechanism whereby relaxin might cause inhibition of contraction. This approach makes it possible to distinguish whether relaxin interferes with the membrane events that regulate intracellular free calcium and hence contraction, or whether the peptide acts at some point beyond that to block the contractile process. The effects of relaxin on the electrical and mechanical activities were studied in isolated longitudinal and isolated circular myometrium because there is evidence that the electrical events underlying contractions in these two layers are different (Osa & Katase, 1975; Kawarabayashi & Marshall, 1981). The rat was chosen for the present study because in this species the electrical and mechanical activities of both the longitudinal and circular elements have been characterized; the effects of relaxin on uterine motility have been most extensively studied in the rat; the levels of relaxin in the circulation during pregnancy are known for this species (Sherwood, Crnekovic, Gordon & Rutherford, 1980).

Brief accounts of some of these results have been presented previously (Parkington, Chamley & McCance, 1981; Parkington & Chamley, 1982).

METHODS

Virgin Sprague–Dawley rats were ovariectomized under ether anaesthesia and 1 week later were given a single subcutaneous injection of 10 μg oestradiol benzoate in 0·1 ml sesame oil. For most experiments, electrophysiological studies were undertaken 24 or 48 h after oestrogen treatment. In other experiments 1 or 10 mg medroxyprogesterone acetate was injected 3 days after oestrogen priming and the uteri were removed 3–5 days after administration of the progestogen. In experiments in which pregnant rats were also used, day 1 of pregnancy was taken as the day on which copulatory plugs were found in the vagina.

Animals were killed by decapitation and both uterine horns removed. Strips of both longitudinal (15 × 2 mm) and circular (10 × 1·5 mm) muscle were prepared by removing the endometrium and the unwanted muscle layer. A silk thread was tied around one end of each strip. The preparation was then mounted horizontally in an organ bath that was partitioned into three compartments by a pair of large stimulating electrodes placed 10 mm apart in the centre of the bath (Abe & Tomita, 1968). About half of the length of the tissue, including the tied end, was placed between the electrodes in the stimulating compartment and the remainder passed through a hole in one stimulating electrode into the recording chamber where the end was secured to the silicone rubber base of the bath by a pair of entomological pins. The thread passed through a hole in the other stimulating electrode, around a small pulley in the third compartment and thence to an isometric force displacement transducer (Grass FTO3). Thus both electrical and mechanical activities could be recorded simultaneously. (There was no resting tension on these preparations that was influenced by isoprenaline (10^{-7} M), removal of external calcium or hyperpolarization of the tissue.) Tissues were continuously perfused with physiological solution containing (mM): NaCl, 120; KCl, 5; KH_2PO_4, 1; $CaCl_2$, 2·5; $MgSO_4$, 1·2; $NaHCO_3$, 25; glucose, 11; saturated with 95% O_2 and 5% CO_2. The temperature of the solution in the bath was maintained at 37 ± 0·5 °C. Transmembrane potentials were recorded using conventional glass micro-electrodes filled with 2 M-KCl, with resistances of around 60 MΩ. The intracellular recording technique and statistical treatment of results have been described previously (Parkington, 1983). After the physiological experiments, strips were chosen at random and prepared for histology to determine whether any circular muscle was attached to strips of longitudinal myometrium or vice versa. This was never observed.

The relaxin used in this study was the CM-a′ fraction extracted from the ovaries of pregnant sows and described by Chamley, Bagoyo & Bryant-Greenwood (1981). As reported in that communication, potency of the CM-a′ fraction differed in different bioassays. In the mouse interpubic ligament bioassay (Steinetz, Beach & Kroc, 1969) the relative potency was 4680 (95% fiducial limits, 3103–5257) guinea-pig units (g.p.u.)/mg; however, in the rat uterine inhibition bioassay (Wiqvist & Paul, 1958) the relative potency was 8217 (6344–10231) g.p.u./mg. NIH-R-P1 (442 g.p.u./mg) was used as standard in these tests. The extract was stored at −20 °C and reconstituted in physiological solution to a concentration of 10^{-3} g/ml immediately before use. Further dilutions were made from this.

The following drugs were used: β-oestradiol-3-benzoate (Sigma); medroxyprogesterone acetate (Provera, Upjohn); verapamil (Isoptin, Knoll); papaverine HCl (DHA); isoprenaline bitartarate (Sigma); propranolol HCl (Inderal, ICI); phentolamine mesylate (Regitine, Ciba); oxytocin-S (Intervet); prostaglandin $F_{2\alpha}$ (Lutalyse, Upjohn).

RESULTS

Longitudinal myometrium

Strips of longitudinal muscle from rats pre-treated with steroid were only spontaneously active, if at all, for up to 30 min after being placed in the organ bath and thus the effect of relaxin on this parameter could not be studied in these preparations. Current pulses (1·2 s duration) were applied every 2 min to induce electrotonic depolarization of the membrane just sufficient to induce a contraction with every stimulus. Bursts of spike-type action potentials were recorded in response to electrical stimulation. The duration of the burst outlasted that of the stimulus and a similar observation has been reported previously in the longitudinal myometrium of the pregnant rat (Osa & Katase, 1975). The amplitude and duration of the contraction which was associated with a burst of spikes was determined by the duration of the burst (Fig. 1*A*). When relaxin (10^{-8} g extract/ml) was applied to these tissues there was a decrease in the number of action potentials in the burst, with a consequent shortening of the duration of the burst and a decrease in the amplitude and duration of the accompanying contraction. This occurred within about

2 min of introducing the peptide. Within 3–5 min, depression of activity progressed until there was total abolition of the response to the control level of electrical stimulation. Increasing the strength of the stimulus induced a short burst of action potentials which was accompanied by a small contraction (Fig. 1*B*). Between 6 and 10 min after the initial introduction of relaxin to the tissue, and in the continued presence of the hormone, the electrical and mechanical response to electrical stimulation returned and recovery was complete within 8–15 min, despite the

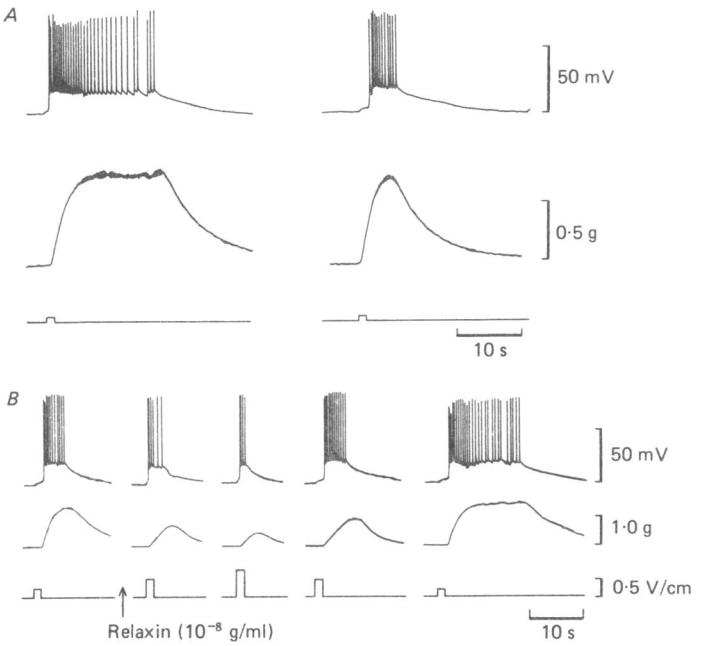

Fig. 1. The effect of relaxin on the electrical activity (upper trace) and mechanical activity (middle trace) evoked by depolarizing current pulses (lower trace) in the longitudinal myometrium of the oestrogen-treated rat. *A*, control solution: note that the duration of contraction depended on the duration of the burst of spike-type action potentials. *B*, in the presence of relaxin (10^{-8} g/ml) the duration of the burst was shortened and hence the amplitude and duration of the contraction was decreased. Activity returned in the continued presence of relaxin and recovery was complete within 8–15 min, despite the continued presence of fresh relaxin in the perfusate.

continuous presence of fresh relaxin in the perfusate. Increasing the concentration of relaxin in the perfusate by 20-fold failed to prevent this break-through in response to a depolarizing current pulse. Furthermore, when there was intermittent but repeated exposure to relaxin, with periods (30–60 min) in control solution interposed, the ability of the peptide to cause inhibition became progressively less until, after three to five exposures, no inhibition was observed. Relaxin had no effect on the resting membrane potential, the space constant or time constant or on the amplitude or maximum rate of rise of the action potentials (Table 1). These findings were common to all preparations of longitudinal myometrium, irrespective of steroid pre-treatment.

In all preparations of longitudinal myometrium obtained from rats up to day 16 of pregnancy, and half ($n = 4$) of those examined on day 17, the effect of relaxin was similar to that observed in longitudinal strips from animals pre-treated with steroids: inhibition was transient and tachyphylaxis was displayed. An example of the effect of relaxin on evoked electrical activity of longitudinal myometrium obtained from a rat on day 15 of gestation is shown in Fig. 2. In tissues from the remaining four rats studied on day 17 of pregnancy, and in all preparations taken from animals from day 18 to term, relaxin was without any detectable effect.

TABLE 1. The effect of relaxin on the resting membrane potential, space constant (λ) and time constant (τ_m) of the longitudinal and circular myometrium of the oestrogen-primed rat. Relaxin had no effect on any of these parameters

	Longitudinal ($n = 11$)			Circular ($n = 17$)		
	Resting membrane potential (mV)	λ (mm)	τ_m (ms)	Resting membrane potential (mV)	λ (mm)	τ_m (ms)
Control	62±1	2·15±0·18	231±22	57±3	2·06±0·47	200±36
Relaxin	62±1	2·14±0·10	244±31	58±2	2·09±0·41	216±27

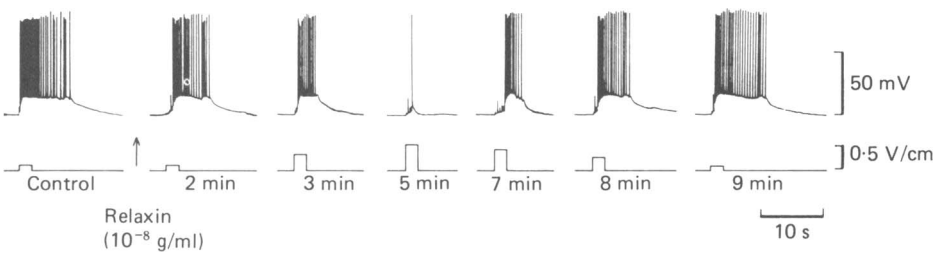

Fig. 2. The effect of relaxin on the electrical activity (upper trace) evoked by depolarizing current pulses (lower trace) in the longitudinal myometrium of the rat on day 18 of pregnancy. Relaxin (10^{-8} g/ml) decreased the number of spikes in a burst. Activity returned within 6–10 min despite the continued presence of fresh relaxin in the perfusate.

Circular myometrium

Non-pregnant

In contrast with the results obtained from longitudinal muscle, strips containing only circular muscle fibres taken from rats pre-treated with oestrogen ($n = 53$) or oestrogen and the progestogen ($n = 11$) were always spontaneously active. The differences in steroid pre-treatment did not have any distinguishable effect on electrical or mechanical activity. Again, in contrast with the longitudinal layer, the action potentials recorded from circular muscle taken from rats pre-treated with steroid consisted of an initial spike or a short burst of spikes followed by a prolonged plateau of depolarization (Fig. 3). These were similar to those observed previously in this muscle layer obtained from pregnant rats (Osa & Katase, 1975; Kawarabayashi & Marshall, 1981). The spikes were always of larger amplitude than those recorded

from the uterus of pregnant animals (compare control in Fig. 3*A*, oestrogen treated, with Fig. 10, pregnant). Action potentials that were indistinguishable from those that occurred spontaneously could be evoked by electrical stimulation (Fig. 3*A*).

When strips of circular muscle were perfused with relaxin (10^{-8} g extract/ml), spontaneous contractions were abolished within 2 min. Electrical stimulation of the tissue evoked contractions that were less than 10% of those in control. Transmembrane recordings revealed that this was the result of a disappearance of the

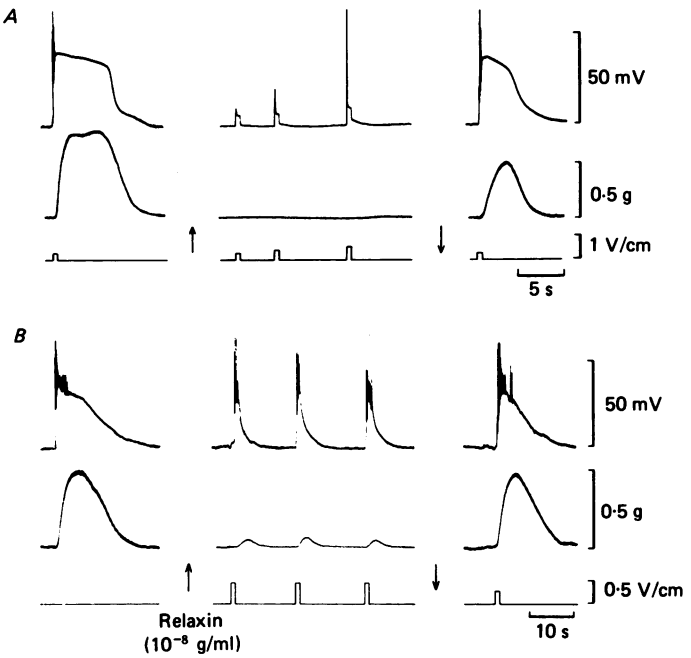

Fig. 3. The effect of relaxin on the electrical activity (upper trace) and mechanical activity (lower trace) of the circular myometrium of the oestrogen-treated rat. The spontaneous (*B*) or evoked (*A*) action potential recorded in this tissue consisted of an initial short burst of spikes followed by a prolonged plateau of depolarization. Relaxin selectively blocked the plateau component leaving intact a single spike (*A*) or a small group of spikes (*B*). The effect was reversed 20–30 min after removal of the peptide. ↓ indicates removal of the peptide from the perfusate.

plateau component of the action potential, whereas the initial spike component remained intact (Fig. 3). Unlike longitudinal muscle, activity of circular strips was depressed by relaxin for as long as the peptide remained in the solution perfusing the preparation. On returning to control solution normal spontaneous activity returned within 20–30 min. Relaxin had no effect on the resting membrane potential or the space or time constants (Table 1).

The inhibitory action of relaxin was not affected by 10^{-7} M-tetrodotoxin, 10^{-6} M-propranolol or 10^{-6} M-phentolamine ($n = 6$ for each treatment).

The effect of other smooth muscle relaxants. Other agents known to inhibit uterine contractions were tested on circular myometrium: isoprenaline, papaverine and verapamil. Fig. 4 shows that isoprenaline and verapamil progressively inhibited both

the spike and plateau components of the action potential; only an electrotonic potential was observed in response to depolarizing current pulses. The effects of isoprenaline were rapidly reversible whereas the effects of verapamil were not. Papaverine selectively blocks the plateau component of the action potential in the ureter of the guinea-pig (Brading, Burdyga & Scripnyuk, 1983). Initially, it decreased the amplitude of the spike component and increased the duration of the plateau of the action potential in the circular myometrium; thereafter, both components were

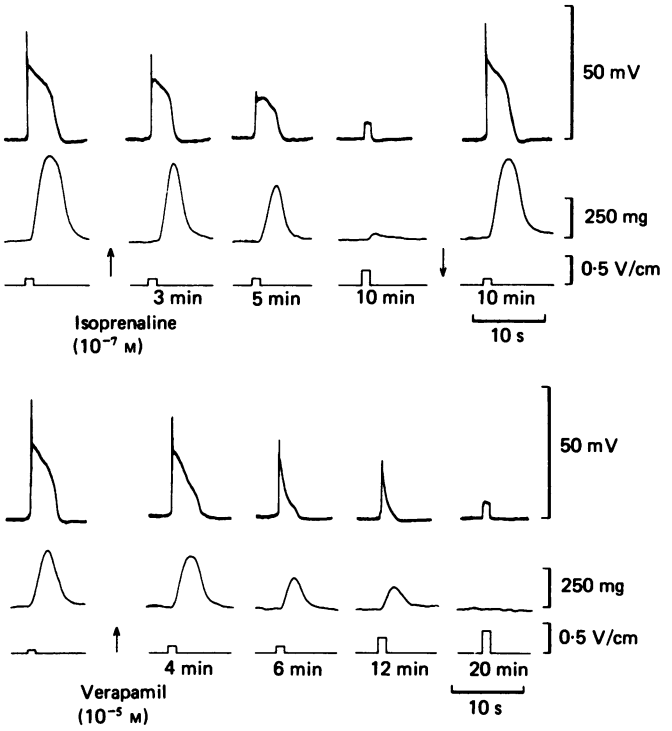

Fig. 4. Isoprenaline and verapamil blocked both the spike and plateau components of the action potential in the circular myometrium of the rat. The effect of isoprenaline was readily reversed whereas that of verapamil was slow and incomplete. ↓ indicates removal of isoprenaline from the perfusate.

abolished (Fig. 5). The effects were rapidly reversible. Two preparations of ureter were impaled with intracellular electrodes and the results of Brading *et al.* (1983) were confirmed.

Oxytocin and prostaglandin $F_{2\alpha}$. Oxytocin increased the amplitude of both the spike and plateau components of the action potential and also increased the duration of the plateau in a dose-dependent manner (Fig. 6*A–C*). Progressively increasing doses of prostaglandin (PG) $F_{2\alpha}$ increased the amplitude and duration of the plateau but did not seem to have any effect on the spike (Fig. 6*D* and *E*). In the presence of either spasmogen the duration of the associated contraction was increased accordingly and the frequency of spontaneous contractions was increased approximately 2-fold. These effects of oxytocin were first observed at a concentration of 10^{-11} M and reached a

maximum effect at 10^{-8} M (Fig. 6*A–C*). Relaxin (10^{-8} g/ml extract) inhibited the response to 10^{-11} and 10^{-10} M-oxytocin but was ineffective when the concentration of the spasmogen was increased further (Fig. 7). The minimum effective concentration of $PGF_{2\alpha}$ was 10^{-10} M and the maximum response was observed at 10^{-8} M (Fig. 6*D* and *E*). Relaxin (10^{-8} g/ml of extract) was effective in abolishing the plateau and reducing the contraction in the presence of 10^{-10} and 10^{-9} M of $PGF_{2\alpha}$ (Fig. 7), but relaxin was ineffective in the presence of 10^{-8} M of the spasmogen. Increasing the concentration of relaxin 20-fold did not alter this result.

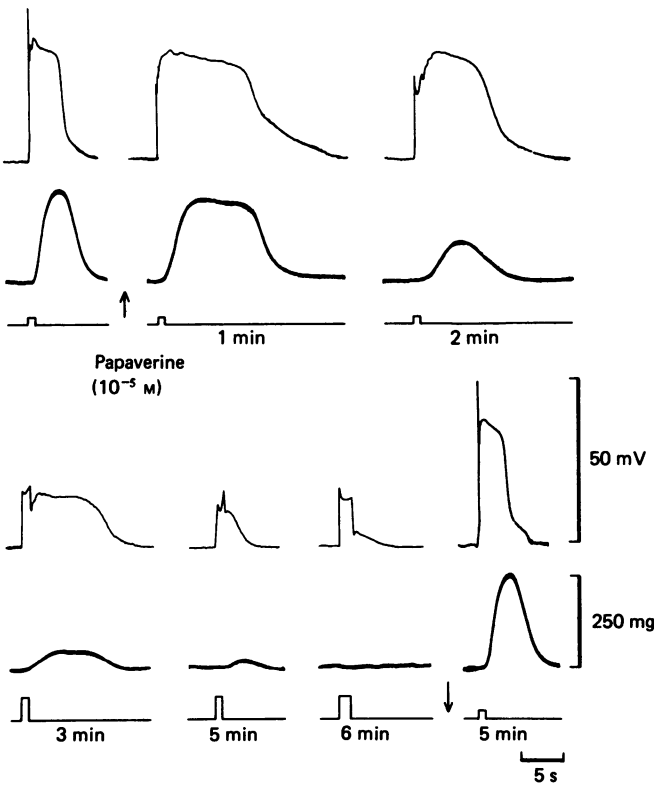

Fig. 5. Papaverine blocked both the spike and plateau components of the action potential in the circular myometrium of the rat. This effect on the spike was more rapid and complete. The effect was reversible. ↓ indicates removal of papaverine from the perfusate.

Calcium. Both oxytocin and $PGF_{2\alpha}$ induce an increase in the transmembrane movement of calcium in longitudinal myometrium (oxytocin: Mironneau, 1976; $PGF_{2\alpha}$: Reiner & Marshall, 1976) but no data are available as to the effect of these spasmogens on the movement of calcium in circular myometrium. In view of the fact that relaxin selectively inhibits the plateau component of the action potential in circular myometrium it was of interest to study the effect of varying the concentration of calcium in the perfusing solution on the action potential in this tissue. The amplitude of the spike component and the resting membrane potential increased as

the concentration of calcium in the perfusate was increased and decreased as external calcium was lowered. Similar observations were reported for the longitudinal myometrium (Marshall, 1962). When studying the effects of varying external calcium concentrations upon the plateau component of the action potential, the concentration of magnesium in the perfusing solution was increased to 5 mM. This ion stabilizes the membrane when the external calcium is lowered, prevents depolarization and thus

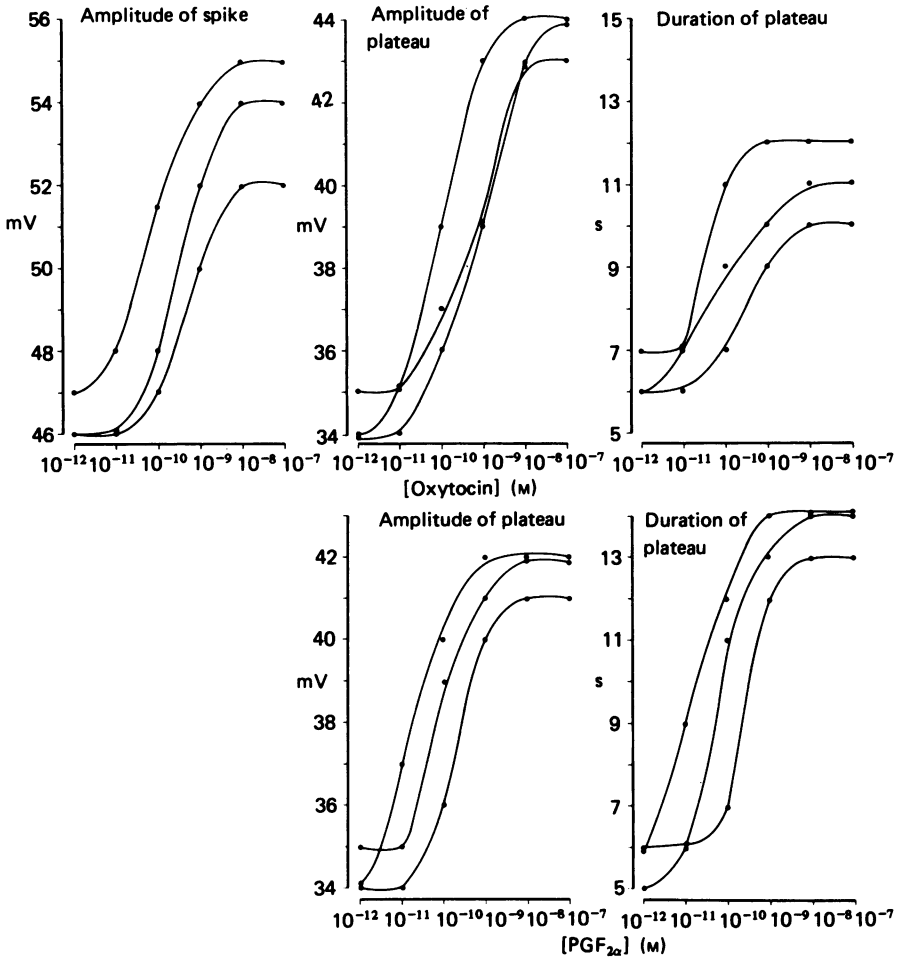

Fig. 6. Oxytocin increased the amplitude of the spike and the amplitude and duration of the plateau, and prostaglandin (PG) $F_{2\alpha}$ increased the amplitude and duration of the plateau component of the action potential in the circular myometrium of the rat. Results from three animals are shown.

eliminates the effects of variations in membrane polarization upon the response under study (Tomita, 1970). In the presence of this concentration of magnesium the characteristics of the action potential were indistinguishable from those observed in control (1·2 mM-magnesium). Fig. 8 shows that the duration of the plateau increased as calcium was increased above 1 mM. When the concentration of calcium in the

perfusate was lowered to below 1 mM the plateau was no longer in evidence and the spike component alone was evoked in response to electrical stimulation (Fig. 8). The duration of the plateau was plotted against the external calcium concentration and the data from three of the seven animals studied are shown in Fig. 9.

There was no effect on the action potential of altering the sodium (to 30 mM) or chloride (to 12 mM) in the perfusing solution.

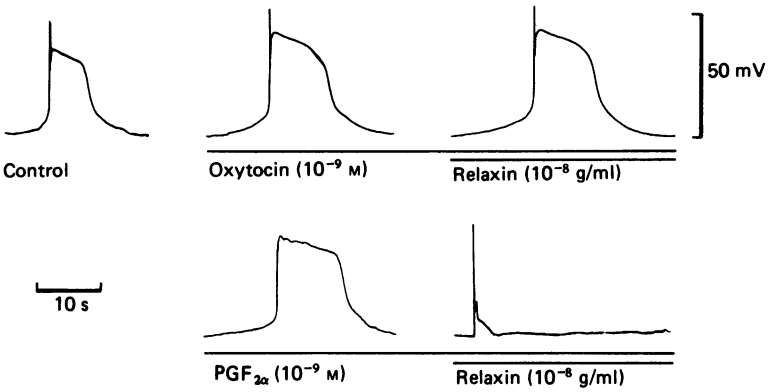

Fig. 7. Relaxin did not affect spontaneous activity induced by 10^{-9} M-oxytocin whereas it blocked the plateau component of the spontaneous action potential induced by 10^{-9} M-prostaglandin (PG) $F_{2\alpha}$ in the circular myometrium of the rat. A spike could be evoked in the latter tissues.

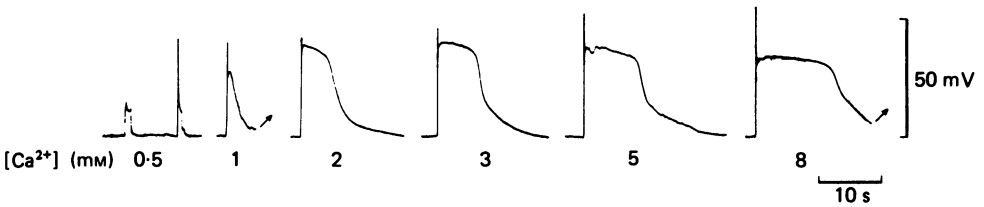

Fig. 8. The effect of altering the calcium concentration in the perfusate on the action potential evoked in the circular myometrium of the rat. Increasing calcium increased the amplitude of the spike and the duration of the plateau. There was no consistent effect on the amplitude of the plateau. The arrow indicates loss of impalement.

Pregnancy

Plateau-type action potentials occur spontaneously in strips of circular myometrium up to day 20–21 of pregnancy in the rat, this type of electrical activity giving way to bursts of simple spikes during the final 24–36 h before parturition (Kawarabayashi & Marshall, 1981; present study). In the present study the effects of relaxin on circular myometrium were examined in fifty rats, from day 13 of gestation to just before parturition. Relaxin inhibited spontaneous electrical and mechanical activity and abolished the plateau component of the action potential in response to electrical stimulation, and reduced the accompanying contraction of all strips in

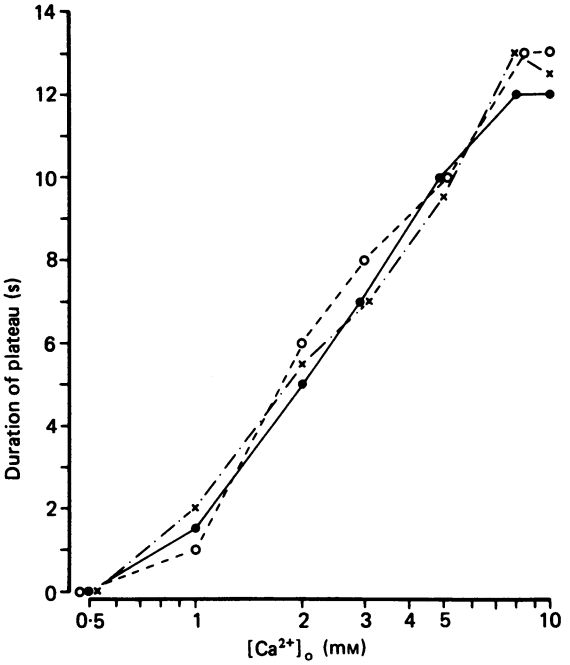

Fig. 9. The duration of the plateau component of the action potential recorded from the circular myometrium of three rats increased linearly with the logarithm of the external calcium concentrations in the range 1–8 mM. When calcium was reduced to below 1 mM the plateau was not evident. There was no further increase in the duration of the plateau when calcium was increased beyond 8 mM.

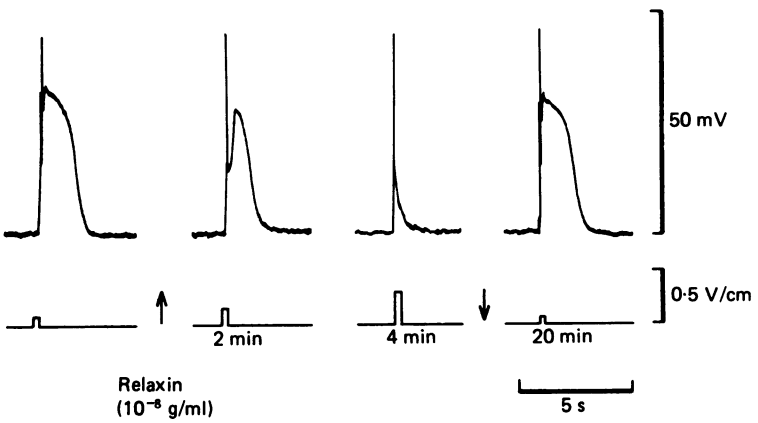

Fig. 10. Relaxin abolished the plateau component of the action potential in the circular myometrium of the pregnant rat while leaving the spike intact. The effect was reversed within 20–30 min of removing the peptide. ↓ indicates removal of the peptide from the perfusate.

which plateau-type action potentials had been recorded prior to exposure to the peptide (Fig. 10). This inhibitory action was observed up to 24–36 h before parturition.

The circular muscle of uterus from rats near term, which displayed bursts of spikes lacking a plateau component in control solution, responded to relaxin in a way similar to strips of longitudinal muscle, e.g. inhibition was transient and tachyphylaxis was evident in response to repeated exposure to the peptide.

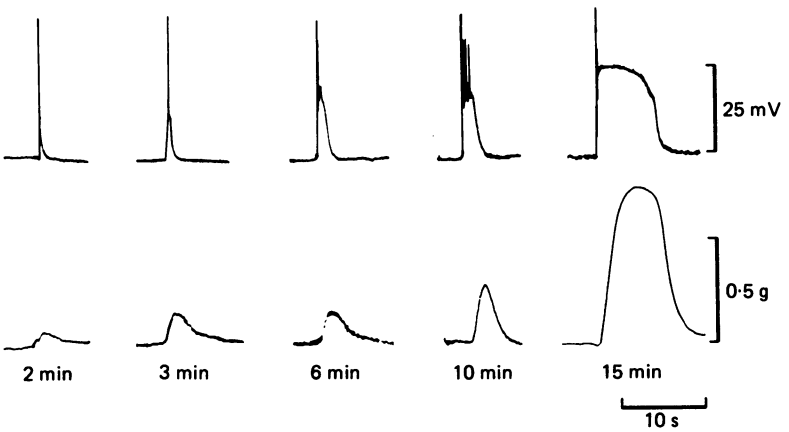

Fig. 11. The spontaneous electrical (upper trace) and mechanical (lower trace) activity recorded from the circular myometrium of the pregnant rat within 15 min of mounting the strip in the organ bath.

It has been shown that the concentration of relaxin appears in measurable amounts around the tenth day of pregnancy in the rat and increases rapidly to reach a plateau of 100–150 ng/ml around day 15 (Sherwood *et al.* 1980). Thus the appearance of spontaneous action potentials with a plateau component up to day 20–21 of pregnancy could have resulted from wash-out of endogenous relaxin during the preparation and equilibration of circular muscle strips. In order to test this possibility, strips from three animals were prepared as quickly as possible and the smooth muscle cells were impaled as soon as the preparation was mounted in the organ bath, without allowing any time for equilibration. Fig. 11 shows the electrical and mechanical activities recorded over the first 15 min. Initially, spikes alone were recorded and these were accompanied by small contractions. After about 3 min the repolarization of the action potential became slower and a plateau component developed in the action potential as time progressed (Fig. 11).

DISCUSSION

This study indicates that relaxin has different actions on the two layers of the uterus. Since the inhibition of the longitudinal layer is transient and tachyphylaxis develops, it seems that the main inhibitory action of the peptide *in vivo* would be on the circular layer. Whether or not this is the case for species other than the rat remains to be explored. Preliminary experiments in this laboratory suggest that

neither layer of the uterus of the guinea-pig is responsive to porcine relaxin (H. C. Parkington, unpublished observations). It has been shown that the plateau component of the action potential, which is characteristic of the electrical activity of the circular myometrium, is most likely to be the target component upon which relaxin acts. It may be useful to bear this in mind when choosing a tissue for the bioassay of relaxin. The observation of Kawarabayashi & Marshall (1981) that the plateau component of the action potential in circular smooth muscle is no longer found at about 24 h before parturition has been confirmed in this study. It seems that when the plateau is evident, for example, in circular muscle from oestrogen-primed, virgin rats, and during pregnancy up to within about 24 h of parturition, relaxin reduces the contraction by abolishing the plateau component of the action potential. However, just prior to parturition, when the plateau component in the circular muscle is replaced by simple spikes, which are always present in longitudinal muscle, the inhibitory effect of relaxin becomes transitory.

Rudzik & Miller (1962*a*, *b*) suggested that relaxin causes the release of noradrenaline, which is responsible for the observed relaxation. In the present study no effect of adrenergic antagonists on relaxin-induced inhibition was observed and this is in agreement with the results of Paterson (1965), Porter, Downing & Bradshaw (1979) and Sanborn, Weisbrodt & Sherwood (1981) who could not confirm an involvement of the adrenergic innervation in inhibition of contraction by relaxin.

The present results may help to clarify some of the apparent contradictions in the literature concerning the ability of relaxin to inhibit contractions elicited with exogenous oxytocin or $PGF_{2\alpha}$. There have been reports that relaxin is effective in preventing contractions induced by oxytocin *in vitro* (Krantz, Bryant & Carr, 1950; Chamley, Bagoyo & Bryant-Greenwood, 1977) and *in vivo* (Porter *et al.* 1979). More recently, Porter, Downing & Bradshaw (1981) have shown that following oestrogen treatment relaxin is effective in inhibiting the response to small doses of oxytocin. Interaction between relaxin and $PGF_{2\alpha}$ with respect to uterine contraction is also unresolved; relaxin did not prevent the contractions induced by $PGF_{2\alpha}$ *in vitro* (Chamley *et al.* 1977) but seemed to be effective *in vivo* (Porter *et al.* 1979, 1981). In the present study relaxin inhibited only the minimum effective dose of oxytocin, but a relatively higher concentration of $PGF_{2\alpha}$ was required to overcome relaxin inhibition. Increasing the concentration of relaxin 20-fold did not change these results.

Intra-uterine balloons record a high frequency of contractions during early pregnancy in the rat. The contractions become less frequent after about mid gestation and cease completely 24–36 h prior to parturition (Fuchs & Poblete, 1970; Fuchs, 1974). Progesterone administration to ovariectomized rats (with or without oestrogen pre-treatment) does not inhibit uterine activity (Fuchs, 1974), and injection of progesterone in pregnant rats, although prolonging pregnancy, causes an increase in uterine activity that is of an uncoordinated nature (Fuchs, 1976). Oestradiol levels increase with the final 24–36 h before parturition in the rat (Yoshinaga, Hawkins & Stocker, 1969) and it has been shown that this steroid can exert an inhibitory action upon uterine contractions (Fuchs, 1976; Downing, Lye, Bradshaw & Porter, 1978; Porter, 1982). However, since the levels of oestrogen in the circulation are undetectable prior to the last 24–36 h before parturition, and progesterone seems to be ineffective

in achieving uterine quiescence, another factor or factors must be responsible for the relatively low level of activity of the uterus from mid gestation up to the last 24–36 h before labour. It is known that the levels of relaxin in the circulation increase from about day 10 of pregnancy onwards in the rat (Sherwood *et al.* 1980). It has been shown in the present study that this peptide is capable of abolishing the plateau component of the action potential that is observed at this stage of pregnancy, thus reducing the contractile response to less than 10%.

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