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EFFECTS OF RITODRINE, A β_2-ADRENOCEPTOR AGONIST, ON SMOOTH MUSCLE CELLS OF THE MYOMETRIUM OF PREGNANT RATS

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1 Effects of ritodrine on the electrical and mechanical activities of the myometrium of pregnant rats were investigated in relation to cyclic adenosine 3',5'-monophosphate (cyclic AMP) production and the actions of isoprenaline.

2 In the longitudinal muscle cells of the 22nd day of gestation, ritodrine ($> 10^{-8}$ M) hyperpolarized the membrane, reduced the membrane resistance and suppressed the amplitude of contraction. Increased concentrations of ritodrine ($> 10^{-7}$ M) suppressed the generation of spikes and increased the amount of cyclic AMP produced. These actions of ritodrine were much the same as those of isoprenaline but the dose-response curves of all parameters were shifted to the right.

3 The effects of ritodrine on the circular muscle cells at the 22nd day of gestation were similar to those observed in the longitudinal muscle cells.

4 In the circular muscle cells at the 17–19th days of gestation, ritodrine produced neither a hyperpolarization nor an increase in cyclic AMP production (up to 10^{-5} M) but did reduce the plateau potential and did relax the tissue ($> 10^{-7}$ M).

5 In the longitudinal muscle cells, during the 17–19th days of gestation, the responses of the tissues were similar to those observed in circular muscles on the 22nd day of gestation. These actions of ritodrine were suppressed by propranolol (10^{-6} M).

6 These results indicate that ritodrine is a β-adrenoceptor agonist and its mechanism of action similar to that of isoprenaline. However, the potency of ritodrine was approximately 100 times less than that of isoprenaline.

Introduction

In the rat myometrium, the longitudinal muscle cells possess mainly β-adrenoceptors, while in the circular muscle cells, α-adrenoceptors are mainly present during middle stage of gestation. However, at the last stage of gestation, β-adrenoceptors in the circular muscle cells are apparent (Kawarabayashi & Osa, 1976; Osa & Watanabe, 1978). Kishikawa (1981) confirmed the above observations on the rat myometrium and further investigated the effects of catecholamines on the myometrium before, during and after delivery in relation to the content of cyclic adenosine 3',5'-monophosphate (cyclic AMP). He reported that in the longitudinal muscles, catecholamines activated chiefly the β-adrenoceptors during gestation, but activation of the α-adrenoceptors was seen during and after delivery, while in the circular muscles, α-adrenoceptor dominance altered to β-adrenoceptor dominance at the last stage of gestation, and then altered again to α-adrenoceptor dominance during and after delivery. In both muscle cells, increases in the cyclic AMP content and activation of the β-adrenoceptors by application of catecholamines appeared simultaneously.

Various β-adrenoceptor stimulants have been used clinically to inhibit uterine contraction and prevent premature labour. However, the use of many of these drugs has been restricted due to cardiovascular side effects such as tachycardia and hypotension. Characterization of β-adrenoceptors in the uterine muscles of some experimental animals revealed that they are dominantly β_2-adrenoceptors (Lands, Luduena & Buzzo, 1967; Larsen, 1979). Ritodrine hydrochloride (ritodrine) is a selective β_2-adrenoceptor agonist and has been effectively prescribed for clinical purposes (Siimes & Creasy, 1976; Caritis, Edelstone & Mueller-Henbach, 1979). However, basic and detailed research on the action of ritodrine on the myometrium of small animals has not been described.

The present experiments were carried out to investigate the effects of ritodrine on the electrical and mechanical properties and cyclic AMP production in the rat myometrium during gestation (17–19th and 22nd days of gestation) and the effects of this agent were compared with those of isoprenaline.

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Methods

Pregnant WKA rats were used. The first day of pregnancy was estimated by presence of the sperm in the vaginal smear test. The experiments were carried out on the longitudinal and circular muscle layers of the myometrium excised from the rats on the 17–19th days and the 22nd day (the final day) of gestation.

Tissue 1–2 mm in width and 5–10 mm in length was dissected from longitudinal or circular muscle strips and mounted in a partition chamber with a volume of 2 ml; superfusion was carried out at a rate of 3 ml/min at a temperature of 35–36°C using a thermo-unit (Kishikawa, 1981). To record the membrane properties of the myometrium, we used a microelectrode consisting of a glass capillary filled with 3 M KCl. The resistance of the electrode measured by d.c. current ranged between 40–90 MΩ. To measure the membrane resistance from the myometrium, the partition stimulating method described by Abe & Tomita (1968) was used. For simultaneous recording of electrical and mechanical activity, one end of the tissue was connected by a silk thread to the mechanotransducer isometrically. The microelectrode was inserted from the serosal side in the case of the longitudinal muscle preparation, and from the mucosal side in the circular muscle preparation. To observe the effects of drugs on the electrically evoked mechanical responses, outward or inward current stimulations of 1 s duration were applied to the preparation, and the effects were determined by % inhibition of the amplitude of contractions during application of the drugs.

To measure the content of cyclic AMP in the tissue, the longitudinal or circular muscle layers were carefully separated under a binocular microscope. The tissue dissected from each layer was kept in the bubbled Krebs solution for 60 min at 37°C. Ritodrine or isoprenaline was applied for the last 5 min during 60 min incubation to observe the effect of drugs on the content of cyclic AMP. To observe the effects of β-adrenoceptor blockade, propranolol 10^{-6} M was applied for the last 10 min of incubation. After the incubation, the tissue was frozen immediately and later homogenized with 6% trichloroacetic acid. The levels of cyclic AMP of the extracts were measured by means of a radioimmunoassay kit (Yamasa Shoyu) as described previously (Kishikawa, 1981).

Modified Krebs solution contained (mM): Na 137.4, K 5.9, Mg 1.2, Ca 2.5, Cl 134.0, HCO_3 15.5, H_2PO_4 1.2 and glucose 11.5 (Bülbring & Kuriyama, 1963), and the solution was equilibrated with a mixture of 97% O_2 and 3% CO_2. The drugs used were isoprenaline hydrochloride (Nikken), ritodrine hydrochloride (N-(P-hydroxyphenylethyl)-4-hydroxynorephedrine: Kissei), propranolol hydrochloride (Sumitomo) and phentolamine mesylate (CIBA-Geigy). Results (membrane potential, % inhibition of contraction and cyclic AMP content) are expressed as the mean ± s.d. of the number of rats examined.

Results

The effects of ritodrine were observed on the myometria of pregnant rats on the 17–19th (mean; 18th) days and 22nd day of gestation, because the pattern of spike generations in the circular muscles changes drastically during the late stage of gestation (Kishikawa, 1981). The resting membrane potentials in longitudinal and circular muscle cells on the 18th day of gestation were -55.7 ± 1.8 mV ($n = 8$ rats; 2–15 penetrations into the cells in one animal) and -45.2 ± 2.3 mV ($n = 4$), respectively, and those on the 22nd day of gestation were -48.5 ± 1.2 mV ($n = 6$) and -44.3 ± 0.7 mV ($n = 5$), respectively. On the last day of gestation, the resting membrane potential of the longitudinal muscle cells was reduced to a value close to that of the circular muscle cells. During the middle stage of gestation, the membrane potential in the longitudinal muscle cells was consistently higher than that of the circular muscles. When the shapes of the action potentials recorded from cells of both muscle layers were compared on the 18th day of gestation, spikes with irregular amplitudes and frequencies appeared as spontaneous bursts of discharges in the longitudinal muscles, but the action potential in the circular muscles was composed of a spike and a subsequently generated plateau potential. On the 22nd day of gestation, spike generation with regular amplitudes and frequencies appeared as a burst of discharges in the longitudinal muscles and as a burst of spikes superimposed on the plateau potential in the circular muscles (Figure 1).

Figure 2 shows the effects of ritodrine on the longitudinal muscle cells on the 22nd day of gestation. Electrical and mechanical activities were recorded simultaneously. Spikes were evoked by electrical stimulation, and as a consequence, the spikes with a uniform amplitude were recorded from the cells of the same muscle layers. To measure the membrane resistance, inward and outward current pulses (1 s in pulse duration) were applied alternately. Application of ritodrine (10^{-8} M) had little effect on the evoked spike and the membrane resistance measured from the change in the amplitude of electrotonic potential, but did reduce the amplitude of the mechanical response evoked by the current pulses. Increases in the concentrations of ritodrine to over 10^{-7} M hyperpolarized the membrane and suppressed the spike generation and contractions produced by outward current pulses. During the hyper-

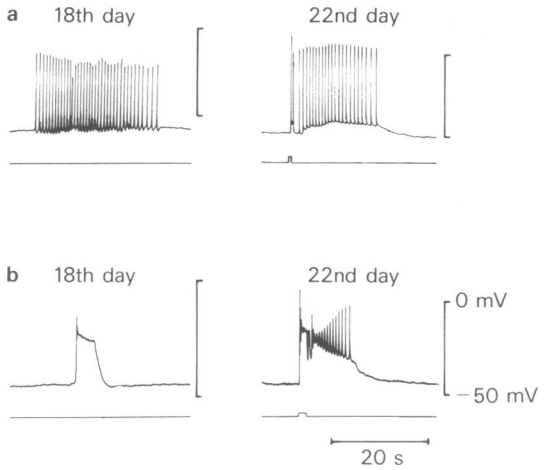

Figure 1 Spontaneous or induced action potentials recorded from the longitudinal (a) and circular (b) muscle cells on the 18th and 22nd days of gestation.

polarization, the membrane resistance was reduced.

When observing the effects of isoprenaline on the membrane and contractile responses, we found that this agent suppressed the electrical and mechanical activities of the longitudinal muscles on the 22nd day of gestation to a greater extent than an equimolar concentration of ritodrine. Figure 3 shows an example of the effects of isoprenaline. Application of 10^{-10} M isoprenaline produced similar effects on the electrical and mechanical activities of the muscle cells as did 10^{-8} M ritodrine. Increased concentrations of isoprenaline (10^{-9} M) hyperpolarized the membrane, reduced the membrane resistance and suppressed spike activity and mechanical responses. These effects of isoprenaline (10^{-9} M) corresponded to those seen with 10^{-7} or 10^{-6} M ritodrine. The potency of isoprenaline was approximately 10^2 higher than that of ritodrine.

To investigate further the effects of ritodrine and isoprenaline on the membrane resistance of the longitudinal muscle cells of rat myometrium on the 22nd

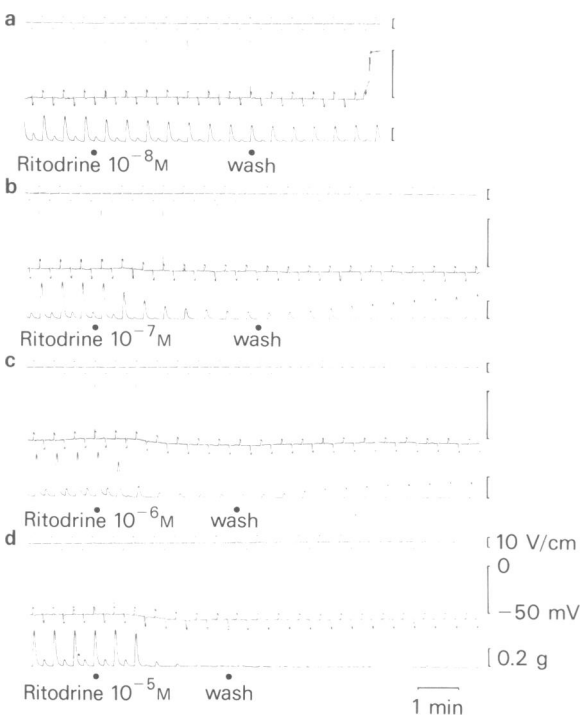

Figure 2 Effects of ritodrine (10^{-8} M–10^{-5} M) on the electrical (middle trace) and mechanical (lower trace) activities in the longitudinal muscle layers of the myometrium (22nd day of gestation). Dots in the figure indicate application and removal of the drug. Inward and outward current pulses (duration; 1 s) were applied alternately (upper trace).

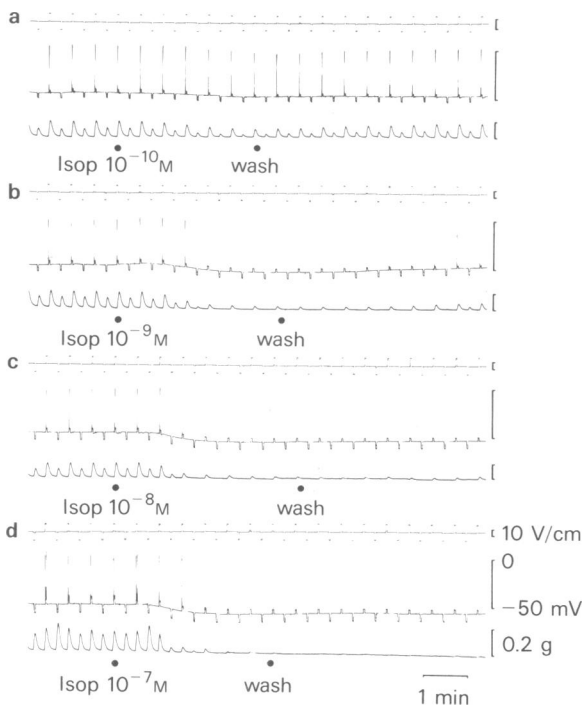

Figure 3 Effects of isoprenaline (10^{-10} M–10^{-7} M) on the electrical (middle trace) and mechanical (lower trace) activities in the longitudinal muscle layers of the myometrium (22nd day of gestation). Inward and outward current pulses (duration; 1 s) were alternately applied (upper trace).

day of gestation, current-voltage relations were observed in the presence of ritodrine or isoprenaline. For such measurements, the microelectrode was inserted within 0.5 mm of the stimulating electrode (length constant of the tissue was 2.9 mm; Kuriyama & Suzuki, 1976). With application of ritodrine (10^{-6} M), the membrane was hyperpolarized and the membrane resistance estimated from the current-voltage relationship was reduced (0.5 mm distance from stimulating electrode; Figure 4a). Application of 10^{-8} M isoprenaline hyperpolarized the membrane to a greater extent than 10^{-6} M ritodrine and the membrane resistance measured from the current-voltage relationship (0.3 mm distance from the stimulating electrode; Figure 4b) was reduced. Figure 5 shows the effects of ritodrine and isoprenaline on the membrane potential, relaxation of the tissue and cyclic AMP production in the longitudinal muscles of the rat myometrium on the 22nd day of gestation. The effects of these agents were also observed in the presence of propranolol. As shown in Figure 5a, the maximum hyperpolarization induced by isoprenaline was -58.5 ± 2.2 mV ($n = 7$) with application of 10^{-7} M and that by ritodrine was -55.6 ± 1.4 mV ($n = 6$) by 10^{-5} M. In the presence of 10^{-6} M propranolol, 10^{-6} M isoprenaline hyperpolarized the membrane to -54.8 ± 2.1 mV ($n = 4$) but no hyperpolarization occurred with application of 10^{-5} M ritodrine (control: -49.0 ± 2.0 mV, $n = 4$; 10^{-5} M ritodrine with 10^{-6} M propranolol: -48.5 ± 0.9 mV, $n = 4$). As shown in Figure 5b, contractions of the tissue generated by electrical stimulation in Krebs solution were normalized as a relative tension (zero inhibition). When the relaxations of the tissue induced by isoprenaline and ritodrine were compared with the change in the membrane potential, about 90% relaxation was observed on application of 10^{-9} M isoprenaline, at a membrane potential of -51.8 ± 2.9 mV ($n = 7$). Such relaxation was observed with application of 10^{-6} M ritodrine at the membrane potential of -55.0 ± 1.0 mV ($n = 6$). For a 50% inhibition of the contraction by application of isoprenaline or ritodrine, a dose of 2×10^{-10} M or 5×10^{-8} M respectively was required. In the presence of propranolol (10^{-6} M), application of 10^{-7} M isoprenaline relaxed the tissue to about 50% of the control value with a slight hyperpolarization of the membrane to -49.6 ± 0.7 mV ($n = 4$), and with application of 10^{-6} M isoprenaline, the tissue relaxed completely at a membrane potential of -54.8 ± 2.1 mV ($n = 4$). On the other hand, following the application of 10^{-5} M ritodrine with 10^{-6} M propranolol, the relaxation was only 13% of maximum with little hyperpolarization of the membrane, as was observed after application of 10^{-8} M isoprenaline with 10^{-6} M propranolol. These results indicate that hyperpolarization of the membrane was usually accompanied by relaxation of the tissue; how-

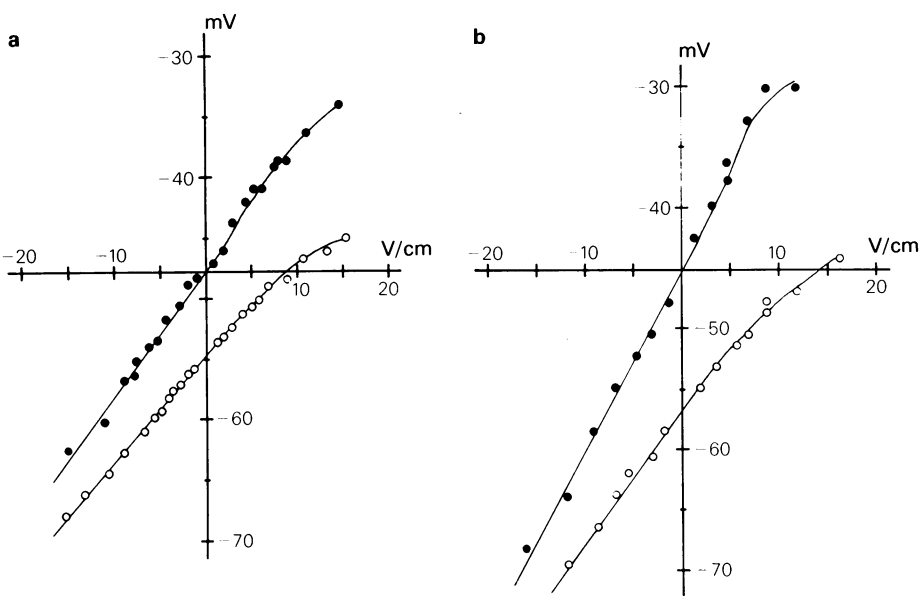

Figure 4 Current-voltage relationships of the longitudinal muscle layer of the myometrium (22nd day of gestation) observed before and during application of 10^{-6} M ritodrine (○) (a) and 10^{-8} M isoprenaline (○) (b); (●) control. Duration of a current pulse was 1.0 s and the recording electrodes were placed in the same cells at a distance of 0.5 mm (a) and 0.3 mm (b) from the stimulating electrode.

ever, the relationship between the degree of the relaxation and hyperpolarization did not always correspond. Production of tissue cyclic AMP by application of isoprenaline or ritodrine was increased but the minimum concentration of isoprenaline or ritodrine required to increase cyclic AMP production was much higher than that which produced hyperpolarization or relaxation of the tissue (Figure 5c); i.e. the concentration of cyclic AMP increased from 0.80 ± 0.22 to 1.81 ± 0.58 pmol/mg wet weight ($n = 4$ animals: 2–6 samples were prepared from one animal) with 10^{-9} M isoprenaline and this concentration was over 10 times higher than that which produced the hyperpolarization or relaxed the tissue. Much the same tendencies were also observed with the application of ritodrine, i.e. the minimum concentration of ritodrine required to increase cyclic AMP production was 10^{-6} M (from 0.70 ± 0.28 to 2.33 ± 1.30 pmol/mg wet weight, $n = 9$). In the presence of propranolol (10^{-6} M), ritodrine did not increase cyclic AMP production up to a concentration of 10^{-5} M, yet 10^{-6} M isoprenaline increased the production (3.58 ± 0.65 pmol/mg wet weight, $n = 4$). This amount of cyclic AMP production corresponded to that produced by 10^{-8} M isoprenaline in Krebs solution and under both conditions, 10^{-8} M isoprenaline in Krebs solution and 10^{-6} M isoprenaline with 10^{-6} M propranolol, the generation of contraction all but ceased (Figure 5).

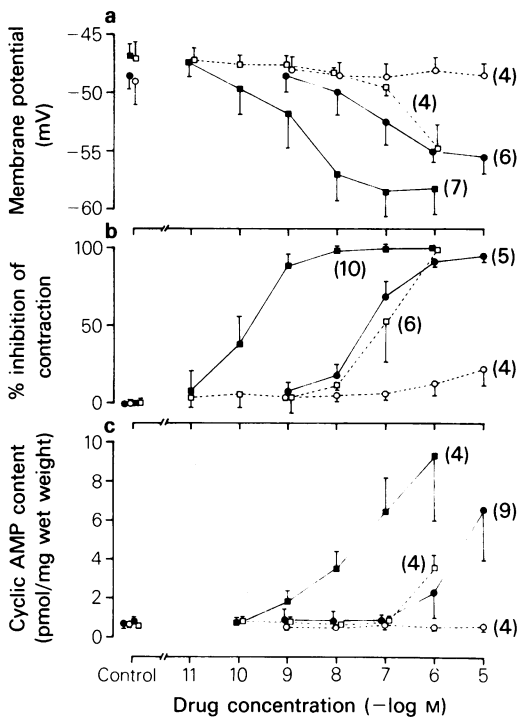

Figure 5 Effects of ritodrine and isoprenaline on the membrane potential (a), mechanical response (b) and tissue content of cyclic AMP (c) in the longitudinal muscles of the rat myometrium (22nd day of gestation) with and without propranolol (10^{-6} M): (●) ritodrine; (○) ritodrine with propranolol 10^{-6} M; (■) isoprenaline; (□) isoprenaline with propranolol 10^{-6} M. Contraction of the tissue was evoked by electrical stimulation (duration, 1 s), and the % inhibition of the amplitude during application of the drugs compared with control contraction is illustrated. Each value is mean of the examined animals (number of rats in parentheses) with s.d.

The effects of ritodrine on the membrane potential, contraction and production of cyclic AMP observed in the circular muscle cells of the 22nd day of gestation were slightly weaker in comparison to those in the longitudinal muscle cells (Figure 6a). The minimum concentration of ritodrine required to produce hyperpolarization was 10^{-7} M (from -44.3 ± 0.7 mV to -46.7 ± 1.3 mV, $n = 5$ rats) and to increase cyclic AMP production was 10^{-6} M (from 0.51 ± 0.5 pmol/mg wet weight to 1.15 ± 0.67 pmol/mg wet weight, $n = 9$). However, ritodrine relaxed the tissue in concentrations over 10^{-7} M. This means that the tissue relaxed under conditions of no change in the amount of cyclic AMP production. To avoid the activation of α-adrenoceptors in the circular muscle, phentolamine (10^{-6} M) was added during application of ritodrine (10^{-6} M); however, no change in the membrane potential was observed. Figure 6b shows the effects of ritodrine on the electrical and mechanical properties of circular muscle cells on the 22nd day of gestation. Outward and inward current pulses of the same intensity were applied alternately. Application of 10^{-6} M or 10^{-5} M ritodrine hyperpolarized the membrane, reduced the amplitude of electronic potential and suppressed the spike generation evoked by outward current pulses. The contraction evoked by current pulses also reduced the amplitude, as expected from changes in the membrane property.

When the effects of ritodrine on the membrane potential, relaxation of the tissue and cyclic AMP production were observed in the longitudinal muscle cells during the 17–19th days of gestation (Figure 7a), the hyperpolarization and relaxation of the tissue induced by ritodrine were similar to those observed in the longitudinal muscle cell on the 22nd day of gestation, yet the increase in the amount of cyclic AMP was much less (after application of 10^{-5} M ritodrine, 2.62 ± 1.83 pmol/mg wet weight on the 17–19th days of gestation, $n = 8$, vs 6.58 ± 2.69 pmol/mg wet weight on the 22nd day of gestation, $n = 9$). However, in the circular muscle cells during the 17–19th days of gestation, ritodrine had little effect on the membrane potential and pro-

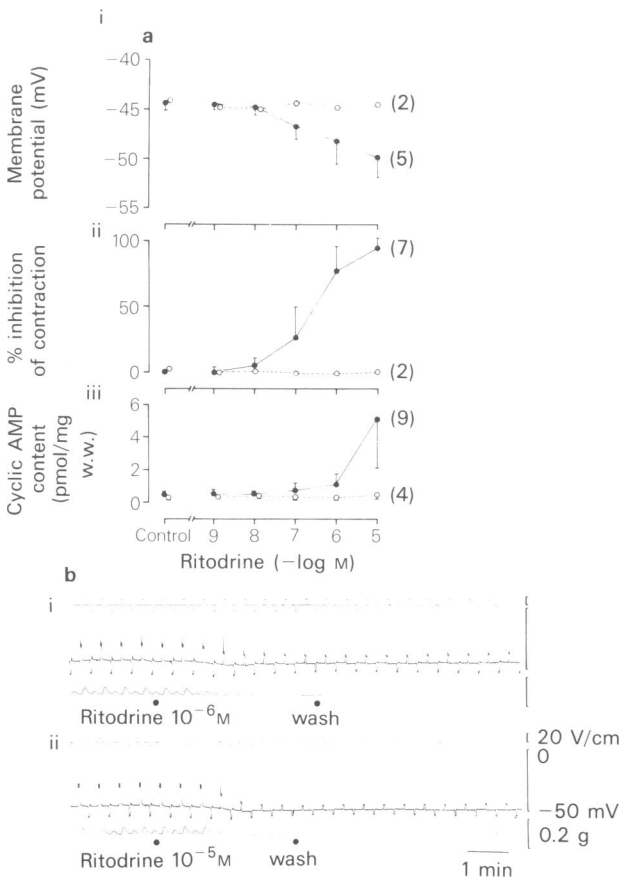

Figure 6 (a) Effects of ritodrine on the membrane potential (i), contraction (ii) and cyclic AMP content (iii) in the circular muscles of the 22nd day of gestation with and without propranolol (10^{-6} M): (●) ritodrine alone; (○) ritodrine with propranolol. Each value is mean of the examined rats (number in parentheses) with s.d. (b) Effects of ritodrine (10^{-6} and 10^{-7} M) on the electrical (middle trace) and mechanical (lower trace) activities recorded from the circular muscle cells of the 22nd day of gestation. Inward and outward current pulses were applied alternately (upper trace, 1 s duration).

duction of cyclic AMP, up to a concentration of 10^{-5} M (Figure 7b). Ritodrine ($> 10^{-7}$ M) relaxed the circular muscle cells during the 17–19th days of gestation to a lesser extent, than it did the longitudinal muscle cells. Application of propranolol (10^{-6} M) inhibited these actions of ritodrine in both the longitudinal and circular muscle cells, at this stage of gestation, although the relaxation persisted to some extent with application of 10^{-5} M ritodrine, in the presence of 10^{-6} M propranolol.

Effects of ritodrine on the electrical properties of longitudinal and circular muscle cells during the 17–19th days of gestation are shown in Figure 8. The spikes were produced by application of outward current pulses in the longitudinal muscles and they were completely abolished by 10^{-6} M ritodrine (Figure 8a). On the other hand, in the circular muscles, the spike was followed by the plateau potential and ritodrine (10^{-6} M) reduced the plateau duration with little effect on the spike amplitude. When the concentration was increased to 10^{-5} M, the spike ceased and there was no hyperpolarization in this case (Figure 8b). In contrast with the longitudinal muscle, cells from the circular muscle layer during the 17–19th days of gestation contracted with little relation to the spike activity, presumably due to de-synchronization of electrical and mechanical activations. Reduction in the duration of the plateau potential accompanied a reduction in the amplitude of the contraction in the circular muscle cells during this stage of gestation. Furthermore, the responses of the circular muscle cells to β-adrenoceptor agonists were weak, probably due to poor distribution of the β-adrenoceptors during the middle stage of gestation.

Discussion

Ritodrine hyperpolarized the membrane, reduced the membrane resistance, increased cyclic AMP production and relaxed the tissue in the myometrium of the pregnant rat. These actions of ritodrine were similar to those of isoprenaline but its potency was about 100 times less than that of isoprenaline. The actions of both ritodrine and isoprenaline were suppressed by propranolol. These actions are thought to be due mainly to activation of β_2-adrenoceptors, although the presence of β_1-adrenoceptors in the uterus has also been suggested (Lands *et al.*, 1967; Larsen, 1979; Johansson, Andersson & Wikberg, 1980). Increase in the membrane potential and decrease in the membrane resistance produced by ritodrine are postulated to be due to increases in the K-conductance of the membrane, as is assumed for the actions of isoprenaline (Kawarabayashi & Osa, 1976; Osa & Kawarabayashi, 1977). The hyperpolarization of the membrane usually accompanied the relaxation of the tissue, although there was a slight discrepancy between the relaxation and hyperpolarization.

In pregnant sheep, Siimes & Creasy (1976) found that when ritodrine was administrated parenterally, this agent effectively inhibited either spontaneous or oxytocin-induced uterine activity. Ritodrine did cause maternal tachycardia but no significant hypotension. Larsen (1979) also reported that in the myometrium of the pregnant goat and cow, ritodrine caused a dose-dependent reduction of the spontane-

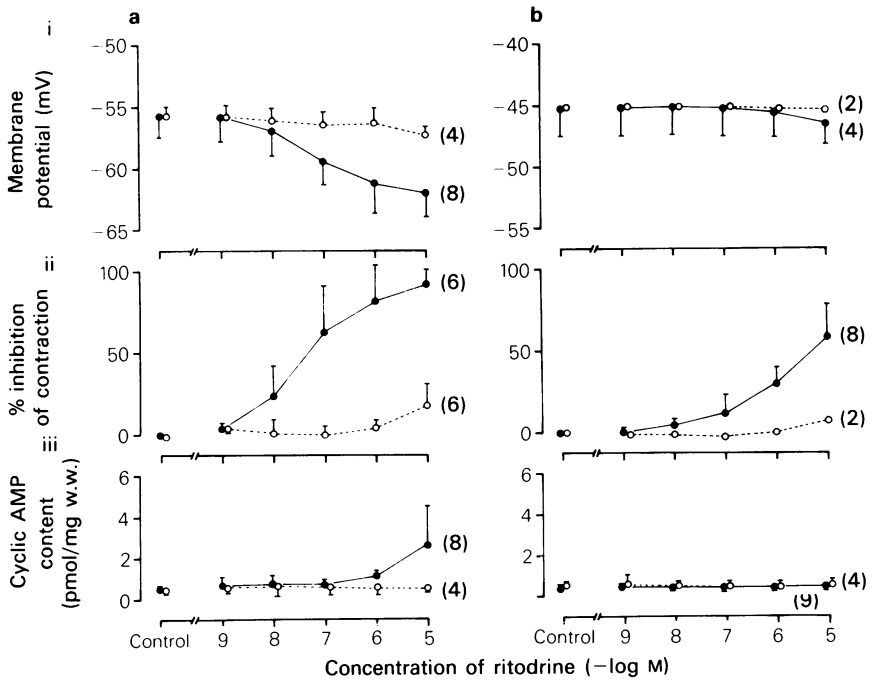

Figure 7 Effects of ritodrine on the membrane potential (i), contraction (ii) and cyclic AMP content (iii) with and without propranolol (10^{-6} M): (●) ritodrine alone; (○) ritodrine with propranolol. (a) Longitudinal muscle cells during the 17–19th days of gestation; (b) circular muscle cells during the 17–19th days of gestation. Each value is the mean with s.d. and the figure in parentheses indicates the number of rats examined.

ous contraction, and the β-adrenoceptors in the myometrium of these pregnant animals belong to the class of β_2-adrenoceptor. The distribution of β-adrenoceptors in the longitudinal and circular muscle cells of rat myometrium differs and this distribution is also affected by the stage of gestation (Kawarabayashi & Osa, 1976; Osa & Watanabe, 1978). In fact, the β-adrenoceptors in the circular muscle cells during the 17–19th days of gestation responded only slightly to ritodrine, as compared to the response noted on the 22nd day of gestation. These actions of ritodrine, as related to β-adrenoceptor activities, were similar to those of noradrenaline or isoprenaline, on the membrane potential, mechanical response and cyclic AMP production during different stages of gestation, in both muscle layers (Kishikawa, 1981). Marked differences in the membrane property, in response to catecholamines, of the circular muscle cells between the 18th and 22nd days of gestation may be mainly, but not solely, due to differences in hormonal domination (Kishikawa, 1981).

Activation of β-adrenoceptors by isoprenaline result in relaxation of muscle tone in various visceral muscles (Bülbring, Ohashi & Tomita, 1981). Theories regarding the underlying mechanism have been put forward by many investigators who used different experimental procedures. When electrophysiological methods were used, action of isoprenaline was postulated to be due to suppression of the spike generating mechanism with or without hyperpolarization of the membrane (Bülbring & Tomita, 1968; Diamond & Marshall, 1969). Using the flux measurement, the isoprenaline action was thought to be related to electrogenic Ca extrusion to extracellular spaces (Marshall & Kroeger, 1973;Bülbring & Den Hertog, 1980). As a result of a biochemical approach, Adelstein, Conti, Hathaway & Klee (1978) presented the hypothesis that an increased amount of cyclic AMP had a direct effect on the contractile protein. This working hypothesis was supported by other investigators who used chemically skinned muscles (Kerrick & Hoar, 1981; Kerrick, Hoar, Cassidy, Bolles & Malencik, 1981; Sparrow, Mrwa, Hofmann & Rüegg, 1981), i.e. activation of catalytic subunits of protein kinase in the presence of cyclic AMP competed with calmodulin's action which modulated the phosphorylation of myosin light chain kinase. Cyclic AMP also accelerated the accumulation of Ca into the storage sites, thus reducing the free Ca in the cell (Ohashi, Ohga & Saito, 1973; Casteels & Raeymaekers, 1979). However, Itoh,

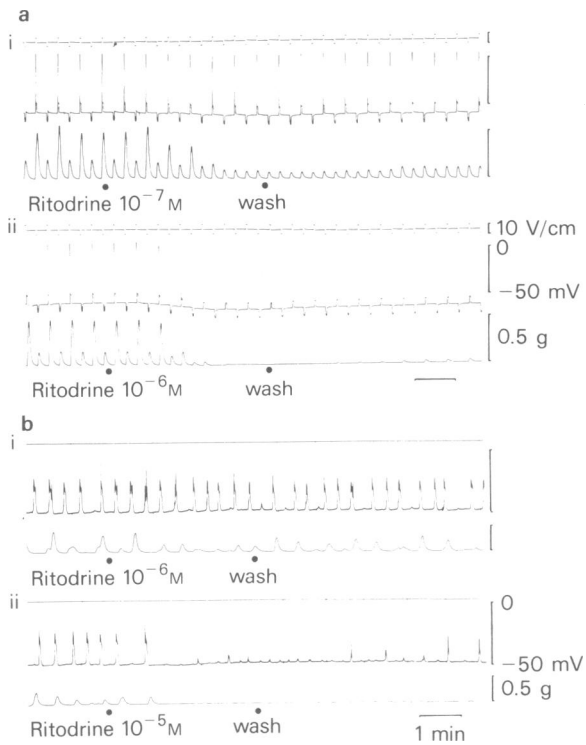

Figure 8 Effects of ridtodrine (10^{-7}–10^{-5}M) on the electrical (middle trace) and mechanical (lower trace) activities recorded from the longitudinal (a) and circular (b) muscle cells of the 18th day of gestation. In the case of (a) inward and outward current pulses were applied alternately (upper trace, 1 s duration). The dots indicate application and removal of the drug.

Izumi & Kuriyama (1981) postulated that an increase in the accumulation of Ca in the storage site induced by cyclic AMP activated the Ca-induced Ca-release mechanism in the storage site and consequently reduced the amount of stored Ca. Since the cell was relaxed, the Ca extrusion from the cell probably coupled with the Ca-induced Ca-release mechanism. Scheid, Honeyman & Fay (1979) postulated that activation of β-adrenoceptors stimulated cyclic AMP-dependent phosphorylation, enhanced Na-K active transport and subsequently reduced intracellular Ca, by an activation of Na-Ca exchange diffusion. These investigations would suggest that activations of β-adrenoceptors by isoprenaline in turn activates adenyl cyclase, increasing the production of cyclic AMP and as a consequence, reduces the free Ca by Ca extrusion to the extracellular spaces and also the Ca accumulation in the storage sites, or may suppress the contractile protein. The present experiments also show that increases in cyclic AMP production are accompanied by the relaxation of the tissue. However, there was a discrepancy between the relaxation and the increase in cyclic AMP production, with application of ritodrine and isoprenaline, i.e. the minimum concentration of these agents required to produce relaxation was lower than that required to induce an increase in the amount of cyclic AMP. Hence relaxation induced by β-adrenoceptor agonists may not be due solely to an increased amount of cyclic AMP production by activation of adenyl cyclase. Further studies are required to elucidate the mechanism of relaxation induced by β-adrenoceptor activation.

In conclusion, ritodrine is a β-adrenoceptor agonist and its mode of action is similar to that of isoprenaline although its potency is low.

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