Br. J. Pharmac. (1968), **34**, 388–407.

Membrane potential and ion content in cat and guinea-pig myometrium and the response to adrenaline and noradrenaline

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1. Cats, virgin and 17 days pregnant, and guinea-pigs, virgin and 14–60 days pregnant, or treated for 1–8 days with oestradiol + progesterone, were used. The response of the uterus to adrenaline and noradrenaline was observed and, in pieces from the same tissues, the resting and active membrane potentials were recorded and the ionic content was determined.

2. Adrenaline and noradrenaline relaxed the virgin cat uterus, adrenaline being 20–100 times more potent *in vivo* and about 10 times or less *in vitro*.

3. Adrenaline and noradrenaline caused contraction of the early pregnant cat uterus, the ratio of potency being about 1.

4. Adrenaline and noradrenaline had a biphasic effect on the guinea-pig uterus in all conditions. The ratio of potency was about 1.

5. The mean membrane potential was 48 mV in virgin cat uterus and 64 mV on the seventeenth day of pregnancy.

6. In guinea-pigs the average membrane potential increased from 38 mV in the virgin uterus to 58 mV on the thirtieth day of pregnancy. A similar increase was produced by eight daily injections of 5 μg oestradiol and, on the last 4 days, additional 1.5 mg progesterone.

7. In the cat, no significant change in K and Na content was observed during pregnancy. The intracellular chloride content, however, rose from 51.5 m-moles/l. fibre water in the virgin uterus to 89 m-moles in the early pregnant uterus. As a result, the calculated chloride equilibrium potential changed from -25 mV in virgin uterus to -11 mV in pregnant uterus.

8. In the guinea-pig no significant change in ion content was observed and the calculated potassium and chloride equilibrium potentials remained both unaltered during pregnancy.

9. In contrast to guinea-pig uterus in all conditions, and to virgin cat uterus, early pregnant cat uterus was not spontaneously active and excess calcium caused no hyperpolarization.

10. The reversal of the uterine response to adrenaline as a result of pregnancy is discussed in relation to the increase of the intracellular chloride content which was only observed in the cat.

In 1906 Dale observed that electrical stimulation of the hypogastric nerve, or the intravenous injection of adrenaline, caused a relaxation of the uterus in the virgin cat but a contraction in the early pregnant cat.

This change of the uterine response during pregnancy can be most clearly demonstrated in the cat. In other species the action of adrenaline varies. Its effect is not only qualitatively different from one species to another, but different authors found the reversal of the uterine response during pregnancy either inconsistent or absent. Miller (1967) has recently summarized the extensive literature on the influence of the hormonal state and of adrenergic blocking agents on the response of the myometrium to catecholamines. The observations show that an inhibitory as well as an excitatory component can be detected in all species. This is either evident in a diphasic response or, if only one phase is observed, the other can be revealed by changing the hormonal state or by applying a specific adrenergic blocking agent.

In the guinea-pig, the usual response of the pregnant as well as the non-pregnant uterus is diphasic. Which of the two predominates depends chiefly on the state of the tissue at the moment of the administration (Hermansen, 1961). In cat uterus the action of adrenaline is reversed from an almost purely inhibitory effect in the virgin uterus, to an almost purely excitatory effect in early pregnancy. Dale already pointed out that in late pregnancy the adrenaline effect becomes diphasic.

The present work was undertaken to find out whether in early pregnant cat uterus special properties could be discovered which distinguished it from virgin uterus and from the uterus of other species. A detailed study of rat uterus has recently been made by Casteels & Kuriyama (1965), who compared the ionic content, the membrane potential and membrane activity of non-pregnant with that of pregnant uterus. They found that there was no significant difference in ion content and they concluded that the increase in membrane potential during pregnancy was mainly due to an increase in K-permeability of the membrane.

We have not carried out a similar investigation of guinea-pig and cat uterus. In the cat, in both conditions, the uterine response to hypogastric nerve stimulation and to intravenous injection of adrenaline and noradrenaline was first recorded *in vivo* and the response to the catecholamines was then again recorded *in vitro*. Experiments on guinea-pig uterus were carried out *in vitro* only. Ion content was measured in parts of the same tissues in which the *in vitro* observations were made.

Several differences were detected between the properties of early pregnant cat uterus and those of other species. In the cat uterus, the membrane potential was highest, spontaneous activity was absent and, in contrast to the other species, excess calcium failed to raise the membrane potential. Moreover, there was a marked increase of the intracellular chloride content during pregnancy. On the basis of these findings a possible explanation of the reversal of the uterine response to adrenaline is discussed.

Methods

Animals

Cats and guinea-pigs were used. We are indebted to the National Institute for Medical Research for letting us have twelve cats from the same breeding colony. Six of these were virgin cats, four 17 days pregnant, and two mated but not pregnant.

Each cat was first used for *in vivo* observations. A spinal preparation was made under ether anaesthesia. After evisceration both hypogastric nerves were prepared for stimulation with shielded electrodes. Both uterine horns were freed from ligaments, tied and cut at the ovarian end and attached to an isotonic lever to record contractions on a smoked drum. The abdominal cavity was filled with warm oxygenated Krebs solution which was kept at 37° C.

The hypogastric nerve was stimulated for 30 sec, using a Grass stimulator, with square pulses of 1 msec duration, at 20/sec. Maximal responses were compared with those to intravenous injections of adrenaline and noradrenaline.

When the observations were complete the cat was bled out and the uterus was removed. Some muscle strips were used for intracellular electrical recording, some for extracellular recording with the sucrose gap method, and the remaining tissue for determining the ionic content.

Twenty-five adult guinea-pigs were used. They were obtained from breeding stock at 250–280 g weight. Groups of five were mated at different dates during a period of 60 days. At the end of this time animals at full term pregnancy weighed 800–865 g. The beginning and progress of pregnancy was estimated by examining vaginal smears and recording the increase in body weight. It was finally checked by measuring the foetuses after the animal was killed. In eight non-pregnant animals and eight animals taken at different stages of pregnancy, the extracellular and intracellular recording as well as the determination of ion content was done in uterine muscle strips from the same animal. Because the amount of tissue was, however, insufficient to obtain significant results for ionic content the remaining animals were used for this purpose only.

An attempt was made to produce changes of uterine muscle properties by hormone treatment comparable with those occurring during pregnancy. Two groups of six immature guinea-pigs, each weighing about 200 g, received subcutaneous injections of 5 μg oestradiol on eight successive days and, in addition, 1.5 mg progesterone on the fifth, sixth, seventh and eighth day. On a given day during this period an animal was taken for the experiment and the uterus was used for intra and extracellular recording. The ion content was not determined.

Solutions

The Krebs solution used in all experiments contained (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.1, $H_2PO_4^-$ 1.2, HCO_3^- 15.5, glucose 11.5; and was aerated with 97% oxygen + 3% carbon dioxide. The pH at 35° C was 7.4. Changes in the ionic composition were made as described by Bülbring & Kuriyama (1963).

Measurements of intracellular ion content

The extracellular space was measured using ^{35}S-labelled ethanesulphonate supplied by the Radiochemical Centre, Amersham. The ethanesulphonate was extracted from the tissue at room temperature and the activity was determined using the scintillation mixture described by Bray (1960).

The water content of the tissues was determined by comparing the wet weight and the dry weight. The latter value was obtained by drying the tissues for 20 hr at 95° C.

The ion content of the samples was determined by flame-photometry using a Zeiss spectrophotometer PMQ II with flame attachment, as described by Casteels & Kuriyama (1965). The intracellular ion content was calculated per litre of fibre water according to Boyle, Conway, Kane & O'Reilly (1941).

Electrophysiological observations

For intracellular recording, pieces of myometrium 3–5 mm wide and 10–15 mm long were mounted in an organ bath kept at 35° C, through which Krebs solution flowed continuously at a rate of 2–3 ml./min. The microelectrodes had a d.c. resistance of 20–50 MΩ and were mounted as described by Woodbury & Brady (1956).

Observations of the effect of catecholamines and adenosine triphosphate on membrane activity and tension were made by extracellular recording using the sucrose-gap method (Stämpfli, 1954 ; Burnstock & Straub, 1958). The tension was recorded with a mechano-electrical transducer valve (RCA 5734). Drugs in solutions of the same ionic content as that of the bathing solution were injected through a narrow polythene tube into the stream of the bathing solution. Each dose was given in a volume of 0.2 cm^3 at a constant rate over a period of 30 sec. The bathing solution flowed at a rate of 2.0 cm^3/min so the drug solution was diluted 5 times. The resulting drug concentration to which the tissue was exposed for 30 sec is stated in the description of results.

Results

Intracellular electrical recording

Cat

Membrane potential

The virgin uterus was spontaneously active, but the discharge was interrupted by silent periods during which the resting membrane potential could be measured. The pregnant uterus was only rarely spontaneously active. In the virgin uterus the membrane potential ranged from 40 to 54 mV, mean 48 mV, S.E. $= \pm 0.86$, $n = 45$. On the seventeenth day of pregnancy the membrane potential was 16 mV higher, ranging from 52 to 75 mV, mean 64 mV, S.E. $= \pm 0.71$, $n = 78$.

Action potential

The typical difference between the virgin and the pregnant uterus was seen in the after-potential of the spike (Fig. 1). In the virgin uterus (a) the spike showed a " negative after-potential," the duration of the falling phase being 0.5–1.2 sec, but in the pregnant uterus (b) the spike showed a " positive after-potential." The after-hyperpolarization had a duration of 0.3–1.2 sec and amounted to 10–30 mV. The maximum rate of rise of the spike was 3–5 V/sec in the virgin uterus and an overshoot potential was not always seen. In the pregnant uterus the maximum rate of rise and the maximum rate of fall was similar, but an overshoot potential was consistently observed ; its maximum was 22 mV and the range from 5 to 22 mV.

Electrical excitability

The virgin uterus had a lower threshold for excitation. At fixed intensity and frequency of stimulation spikes were evoked in the virgin uterus by stimuli of 1–2 msec pulse duration, while in early pregnant uterus 3–5 msec were required.

Figure 2 shows the effect of changing the frequency of stimulation (0.3–1 c/s) on the spike in pregnant uterus. Two different cells are demonstrated. (a) shows a cell with a low membrane potential and high amplitude of the after-hyperpolarization, and (b) shows a cell with a high membrane potential and low amplitude of the after-hyperpolarization. Electrical stimulation at a frequency exceeding 0.5 c/s reduced the spike amplitude and the after-hyperpolarization ; whereas at a frequency of less than 0.5 c/s, the spike amplitude remained the same, but the amplitude of the after-hyperpolarization increased.

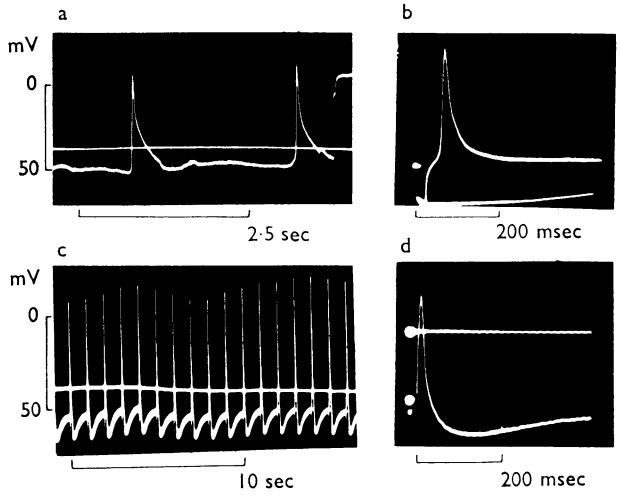

FIG. 1. Cat uterus. Intracellular records of (a) spontaneous, (b) evoked action potentials in virgin, (c) and (d) in 17 days pregnant uterus, recorded on two different time bases. For description see text. (Second trace tension record.)

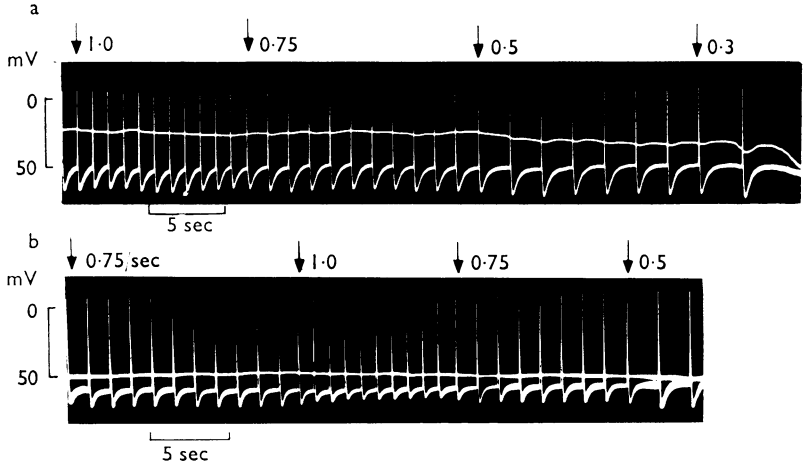

FIG. 2. Pregnant cat uterus. Effects of changing the frequency of stimulation on the amplitude of the spike and the after-hyperpolarization, recorded from two different cells (a and b).

Effect of changing the external ion concentration on the spike activity

Potassium. The depolarization caused by increasing the external potassium concentration was less in the virgin than in the pregnant cat uterus. Figure 3a and b shows the effect of excess potassium (17.7 mM) which depolarized the membrane and abolished the after-hyperpolarization of the spike in pregnant uterus. Figure 3c and d shows the effect of simultaneous treatment with excess potassium (29.5 mM) and excess calcium (12.5 mM). Although the membrane was less depolarized, the after-hyperpolarization was also almost abolished. The maximum rates of rise and fall were considerably reduced (c′) and (d′). Reduction of the external potassium concentration (2 mM) transiently increased the after-hyperpolarization. These observations indicate that the after-hyperpolarization was due to an increased potassium permeability, shifting the membrane potential towards the potassium equilibrium potential.

Chloride. Chloride deficient solution (using ethanesulphonate as substitute) depolarized the membrane. In 67 mM Cl, the membrane potential of pregnant uterus fell from 68 mV to 65 mV ($n=30$), and in 33 mM Cl it was reduced to 56 mV ($n=30$) as shown in Fig. 4. The amplitude of the after-hyperpolarization was, however, increased by the reduction of the external chloride concentration. Thus the absolute values of the potential difference from the crest of the after-hyperpolarization to zero potential remained nearly constant before and after treatment with low

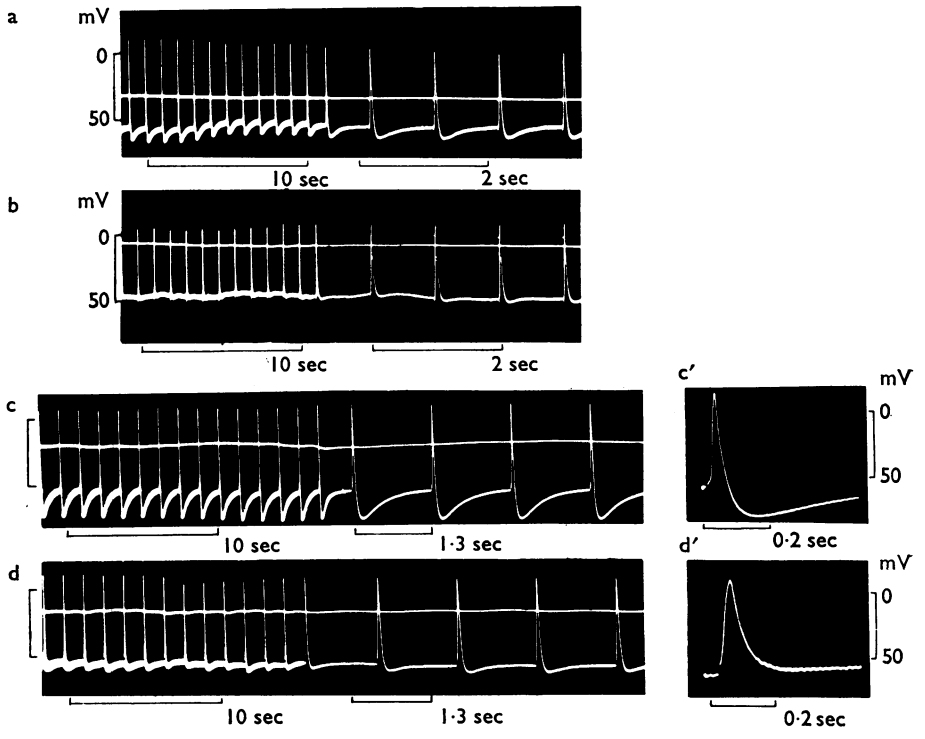

FIG. 3. Pregnant cat uterus. Effect of changing the external K concentration on the after-hyperpolarization following the spike, recorded at different sweep speeds (a) in normal solution, 5.9 mM K, (b) in 17.7 mM K, (c and c′) different cell, in 5.9 mM K, (d and d′) in 29.5 mM K and 12.5 mM Ca.

chloride solution. The observations were made after exposure for 30–50 min to chloride deficient solution. They indicate that the permeability for both potassium and chloride ions contributes to the membrane potential.

Sodium. Low sodium (15.5 mM Na ; replacement by Tris) transiently increased the membrane potential in pregnant uterus and then gradually lowered it. After exposure for 15–20 min to low sodium the membrane potential was lower than before treatment. The overshoot potential and the maximum rates of rise and fall were reduced. The amplitude of the after-hyperpolarization was increased as a result of the lower membrane potential.

Calcium. Excess calcium (7.5 mM) depolarized the membrane and accelerated the spontaneous membrane activity in the virgin uterus ; but this concentration had no effect on early pregnant uterus. A higher concentration of calcium (12.5 mM) increased the membrane potential of the virgin uterus and slowed activity, but caused no hyperpolarization in the early pregnant uterus.

Effect of storage of the tissue at low temperature

It is possible that after prolonged isolation *in vitro* the characteristic behaviour of the myometrium for a specific hormonal domination may change. Some experiments were therefore designed to find out whether the difference in the shape of the spike between virgin and pregnant uterus was due to a change of the membrane properties, or to the presence of a hormone or some other substance affecting membrane permeability, which could diffuse away.

The tissues taken from pregnant uterus were kept at 4° C for 24 hr, 48 hr and 72 hr. Before the experiment, the preparation was transferred to room temperature under well oxygenated conditions for 30 min. It was then set up in the organ bath at 35° C and left for 1 hr. Even after 3 days storage spikes could be evoked by electrical stimulation. After 24 hr storage there was neither a change of

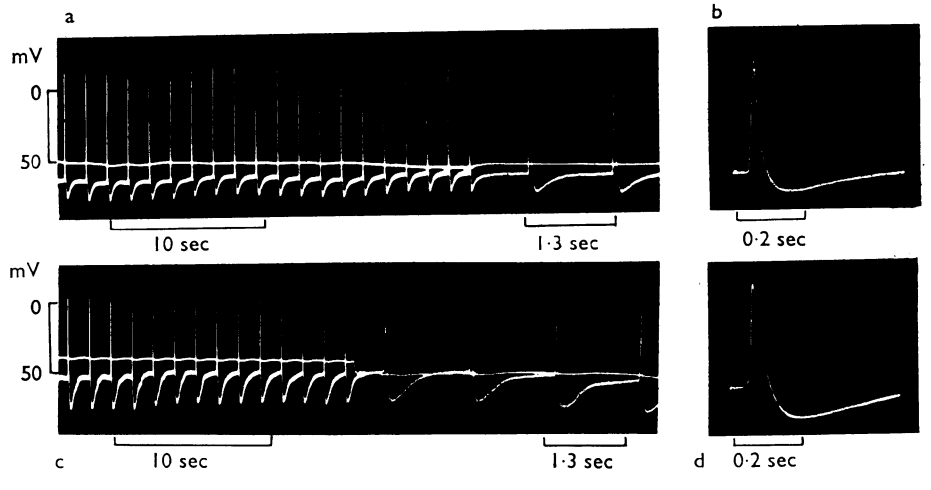

FIG. 4. Pregnant cat uterus. Effect of lowering the external Cl concentration on the after-hyperpolarization following the spike. (a and b) Normal solution, 134.1 mM Cl ; (c and d) 33 mM Cl ; NaCl being replaced by sodium ethane sulphonate. Note different time scales.

membrane potential nor of spike amplitude or shape. After 72 hr storage the membrane potential had fallen from 64 mV to 56 mV, but the shape of the spike was not influenced at all and the after-hyperpolarization was clearly observed. This result may indicate that the change of the spike shape is due to a qualitative change of the membrane itself during pregnancy.

Guinea-pig

Non-pregnant uterus

The muscle was spontaneously active. The membrane potential, measured during silent periods, was between 25 and 45 mV, mean 38 mV. No difference could be observed between the conditions of oestrus and anoestrus. The spikes appeared as bursts of discharges at intervals of 25–30 sec. The number of spikes in a train was 2–23, the frequency 0.6–2.5/sec. In some experiments, after exposure for 2–3 hr to Krebs solution, the spike discharges became continuous.

The spike amplitude varied from 15 to 45 mV. The smaller ones often had a duration of more than 200 msec, and might be " slow potential changes." An overshoot potential was rare and was seen consistently in only one out of eight experiments. The first spike in a train always had the fastest rate of rise, maximum 2–4 V/sec. The shape of the spike was characterized by a slow repolarization phase and plateau formation so that, in a train discharge, successive spikes appeared on the falling phase of the preceding spike (Fig. 5a).

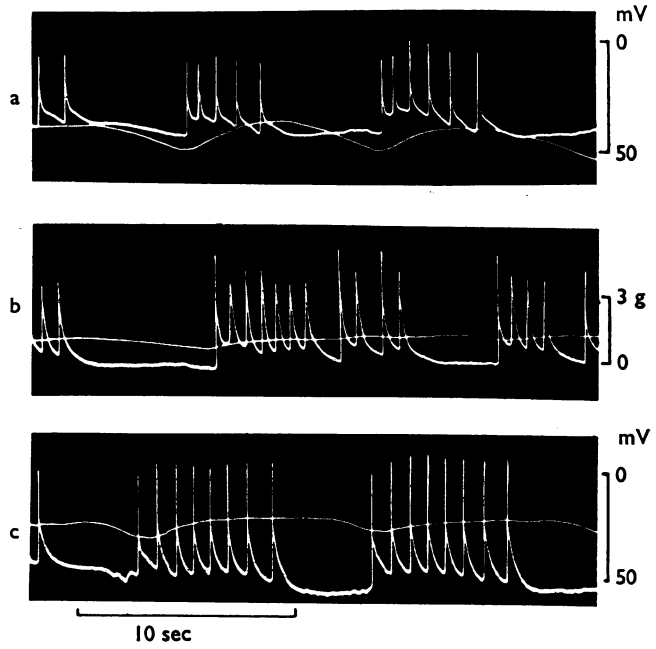

FIG. 5. Mature guinea-pig uterus. Intracellular records of spontaneous activity in (a) non-pregnant, (b) fifteenth day, (c) thirtieth day pregnant uterus.

Pregnant uterus

Figure 5 shows the membrane activity in a non-pregnant uterus (a), on the fifteenth day (b) and on the thirtieth day of pregnancy (c). Spontaneous activity appeared also during pregnancy, with the same shape and number of the spikes in a train. The average membrane potential increased during the course of pregnancy from 38 mV to 58 mV (thirtieth day). The spike amplitude and its maximum rate of rise increased with the increased membrane potential.

Effects of oestradiol and progesterone on the resting membrane potential and the action potential

Immature virgin guinea-pigs were treated for 8 days with daily injections of oestradiol and, for the last 4 days, with additional progesterone totalling four injections. Table 1 shows the change of the uterine membrane potential and the spike amplitude in two groups of six animals and Fig. 6 shows the changes of the membrane activity. After 2 days injection of oestradiol (a) the membrane potential was slightly increased (average 42 mV) but the amplitude of the spontaneous as well as the evoked spike was still low. After 6 days injection of oestradiol and 2 days progesterone (b) the mean membrane potential was 55 mV and the spikes showed an overshoot of 2–8 mV. The spike amplitude and the number of spikes in a train was uniform (Fig. 6b). Simultaneous treatment with oestradiol and progesterone (c) further increased the membrane potential up to a maximum of 60 mV (8 days oestradiol and 4 days progesterone). The spontaneous discharges were interrupted by longer intervals, and sometimes the silent period exceeded 5 min. The shape of the spikes, whether spontaneous (a′ and c′), or evoked by electrical stimulation (c″) was not influenced by hormonal treatment—that is, the spike was always followed by a negative after-potential.

Effect of extracellular stimulation

Casteels & Kuriyama (1965) reported that the threshold to trigger the spike in rat uterus changed in different physiological conditions. The minimum pulse duration required to trigger the spike was therefore measured after hormonal treatment. At constant strength (10V), non-pregnant uterus required 3 msec, after 2 days oestradiol treatment only 1 msec. After 5 days oestradiol and 1 day progesterone, a pulse of 0.5 msec produced a spike (Fig. 6 c″). However, after one day without treatment, following 8 days oestradiol and 4 days progesterone, the pulse duration required to trigger the spike was again 3 msec.

TABLE 1. *Membrane potential and action potential (mV) recorded in guinea-pig uterus after treatment with oestradiol and progesterone on eight consecutive days*

Oestradiol	None	1	2	5	6	7	8	1 day after the last injections
Progesterone	None	None	None	1	2	3	4	
Membrane potential								
Mean	38±2·2	39±2·8	42±2·3	52±1·8	55±1·6	53±2·0	58±3·1	53±3·0
Range	25–45	35–45	35–45	50–55	50–60	45–60	55–60	50–55
Action potential								
Mean	29±3·8	32±5·2	30±2·8	58±3·6	60±2·8	57±2·4	59±3·7	57±2·0
Range	15–45	25–45	21–40	50–65	54–65	50–60	45–65	50–60

Repetitive stimulation of the guinea-pig uterus changed the spontaneous rhythm of membrane activity and when spikes were evoked regularly by extracellular stimulation at a frequency of about 0.5 c/s, the spontaneous activity was completely blocked. At higher frequencies of stimulation (more than 0.5 c/s) the spike amplitude and the duration of the after potential were reduced and the membrane was gradually depolarized (Fig. 6d, e).

Effects of calcium

The guinea-pig uterus was very sensitive to changes in the external calcium concentration. Reduction to half (1.25 mM Ca^{2+}) depolarized the membrane in all conditions. The spike activity quickly deteriorated and stopped. When the external calcium concentration was increased the membrane potential rose. The magnitude of the effect increased with the intensity of hormonal treatment. After 6 days oestradiol and 2 days progesterone, 12.5 mM Ca increased the membrane potential up to 70 mV. In spite of this effect on the membrane potential excess calcium increased the spontaneous spike frequency. This produced a depolarization, so that, during a burst, the train of discharge arose from a lowered potential level. In the experiment shown in Fig. 7 (a, b, c) the discharge became almost continuous, in that shown in Fig. 7 (d, e, f) the number of spikes per train increased but silent intervals became longer.

Effect of potassium

Excess potassium depolarized the membrane and the spontaneous activity became continuous. The spike amplitude and the maximum rate of rise of spike were

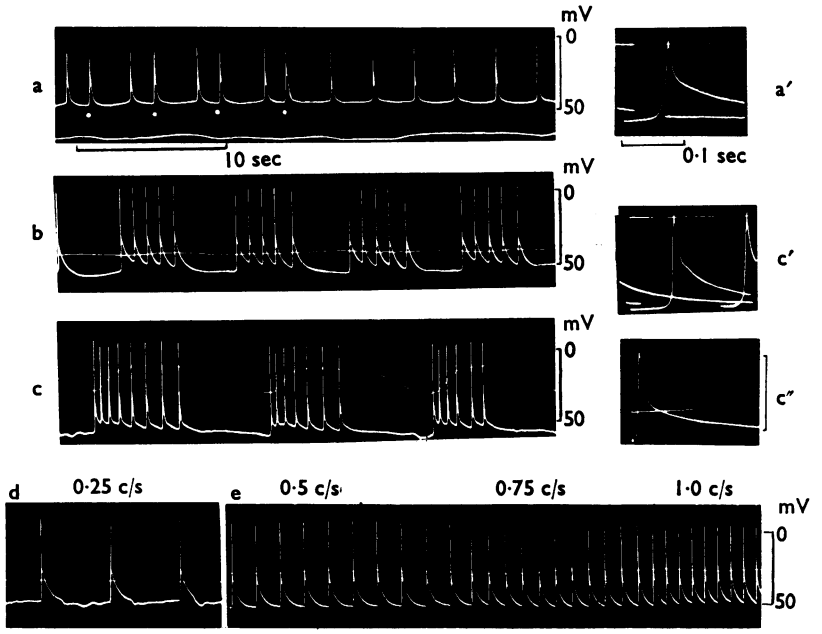

FIG. 6. Immature guinea-pig uterus. Intracellular records of spontaneous (a, a′, b, c, c′) and evoked (c″, d, e) spikes. (a) Untreated control ; (b) after 6 days oestradiol and 2 days progesterone injections ; (c) after 8 days oestradiol and 4 days progesterone injections. For description see text.

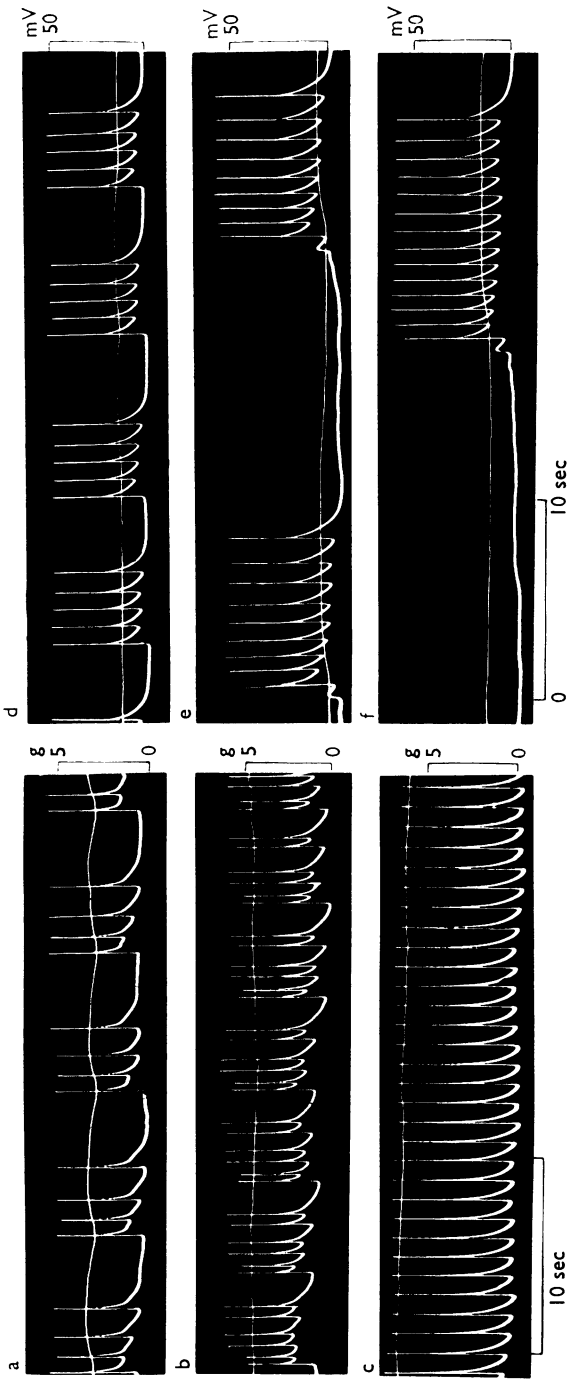

FIG. 7. Guinea-pig uterus. Intracellular records of spontaneous activity. The effect of excess Ca. (a–c) After 5 days oestradiol and 1 day progesterone injections; (d–f) after 6 days oestradiol and 2 days progesterone injections. (a) and (d) in normal solution, 2.5 mM Ca; (b) and (e) in 6.25 mM Ca; (c) and (f) in 12.5 mM Ca. For description see text.

reduced and, at ten times the normal potassium concentration (59 mM), spontaneous activity ceased. The changes produced in the uterus after 7 days oestradiol and 3 days progesterone are shown in Fig. 8.

Ionic content

Cat

The ionic content of the virgin and early pregnant cat uterus after equilibrating the tissues for 90 min in Krebs solution at 36° C is given in Table 2. The intracellular ionic content was calculated per litre of fibre water (Boyle, Conway, Kane & O'Reilly, 1941) from the total content and the ethanesulphonate space. The potassium and sodium content were very similar in the virgin and early pregnant uterus, and therefore the potassium and sodium equilibrium potentials were not significantly different for the two conditions. However, the intracellular chloride content Cl_i was much higher in the early pregnant than in the virgin cat uterus. The calculated chloride equilibrium potentials were -25 mV in virgin uterus and -11 mV in early pregnant uterus.

Guinea-pig

The ionic content of guinea-pig uterus, virgin and at different stages of gestation, in *in vitro* conditions is summarized in Table 3. In contrast to the observations on cat uterus, no significant change in chloride content was observed during pregnancy,

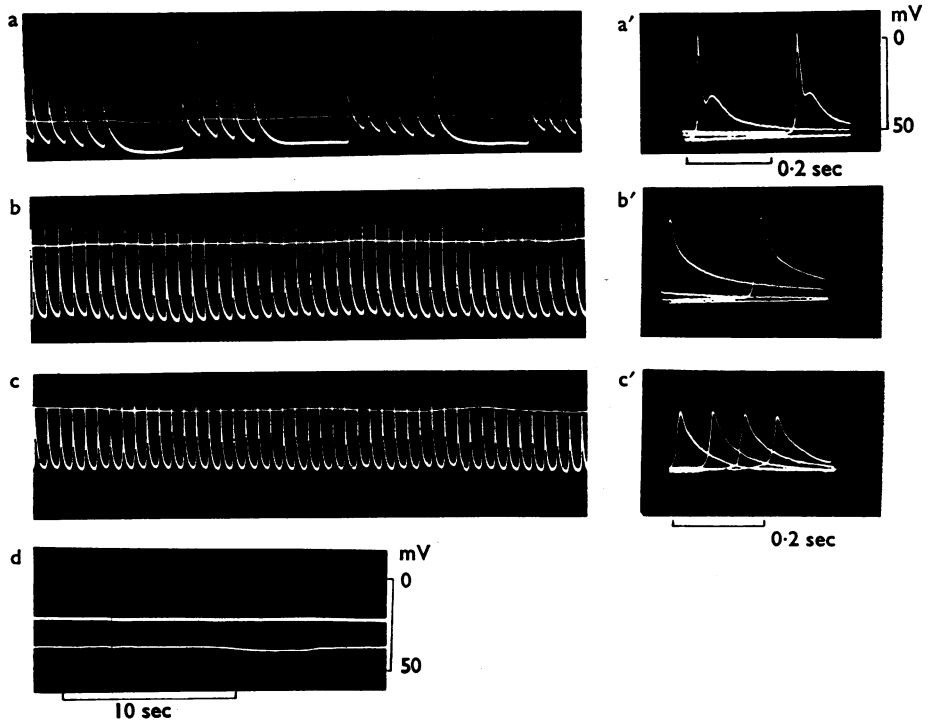

FIG. 8. Guinea-pig uterus after 7 days oestradiol and 3 days progesterone injections. Intracellular records of spontaneous activity. (a, a') In normal solution, 5.9 mM K; (b, b') in 17.7 mM K; (c, c') in 29.3 mM K; (d) in 59 mM K.

and not only the calculated potassium potential but also the chloride equilibrium potential remained unchanged.

Effect of adrenaline and noradrenaline

In vivo *experiments in cats*

In the spinal cat, stimulation of the hypogastric nerve invariably caused relaxation of the virgin uterus and contraction of the early pregnant uterus, as observed by Dale (1906).

In the virgin cat, the dose of adrenaline which produced a relaxation equal to that caused by maximal nerve stimulation was about 2 μg for a cat weighing 2.5 kg, but the dose of noradrenaline required to produce the same effect was 20–100 times larger. The threshold dose of adrenaline varied from 0.2 to 1.0 μg. These observations agree with those of Vogt (1965).

In the pregnant cat, a contraction equal to that caused by maximal nerve stimulation was produced by adrenaline and noradrenaline in the same dose range of 5–10 μg, the threshold for both being about 0.5–1.0 μg. Vogt (1965) also observed that the ratio of doses producing the same contraction was about 1. The contraction produced by adrenaline was sometimes of shorter duration and was followed by some relaxation. In one pregnant cat it was not possible to find a dose of adrenaline

TABLE 2. *Ionic content (m-moles/kg wet weight), extracellular space (ml./kg wet weight), intracellular ion concentration (m-moles/l. fibre water) and equilibrium potentials (mV) in cat uterus after equilibration for 90 min in Krebs solution at 36° C.*

	Virgin cat uterus	Early pregnant cat uterus
K^+	47·3±1·4 (n=36)	52±1 (n=59)
Na^+	95·1±2·1 (n=36)	91±1·2 (n=65)
Cl^-	86·6±2·4 (n=35)	96±0·8 (n=60)
Ethanesulphonate space	534 ±31 (n=9)	519±13 (n=26)
Dry weight/wet weight	18·4±0·6 (n=8)	18·4±0·5 (n=22)
$[K]_i$	157	164
$[Na]_i$	78·5	67·5
$[Cl]_i$	51·5	89
E_K	−88	−88·5
E_{Na}	+15	+19
E_{Cl}	−25	−11

TABLE 3. *Ionic content (m-moles/kg wet weight), extracellular space (ml./kg wet weight), intracellular ion concentration (m-moles/l. fibre water) and equilibrium potentials (mV) in guinea-pig uterus after equilibration for 90 min in Krebs solution at 36° C.*

	Virgin	14 days pregnant	25 days pregnant	40 days pregnant	60 days pregnant
K^+	72·9±1·2 (37)	77±2·5 (15)	79·3±1·5 (12)	81·2±0·6 (15)	82·1±1·7 (10)
Na^+	84·8±2·6 (37)	81·5±2·6 (15)	78·6±1·8 (12)	63·3±1·6 (15)	62·7±1·6 (10)
Cl^-	85·5±1·1 (37)	79·6±2·1 (15)	86±1·3 (12)	78±1·3 (16)	78·1±1·4 (10)
Ethanesulphonate space	487±14 (19)	396±15 (7)	438±29 (6)	365±12 (9)	356±12 (11)
Dry weight/ wet weight	19·8±0·6 (15)			19·5±0·5 (11)	
$[K]_i$	222	183	209	180	178
$[Na]_i$	60	67	51	30	31
$[Cl]_i$	66	65	73	66	67
E_K	−96	−91	−95	−91	−91
E_{Na}	+22	+19	+26	+40	+40
E_{Cl}	−19	−19	−16	−19	−19

which caused a contraction of the same size as that produced by noradrenaline or by nerve stimulation. Over the whole range tested (0.5–20.0 μg), adrenaline produced in this experiment a biphasic response—a small short contraction followed by a larger relaxation.

A biphasic response was also observed in two non-pregnant cats tested following ovulation. These responded to adrenaline with relaxation or a small contraction followed by relaxation (threshold 0.2 μg and 1.0 μg in the two animals) and to noradrenaline with contraction (threshold 5 μg and 10 μg respectively). The dose required to match the contraction produced by maximal nerve stimulation was 10 μg and 50 μg noradrenaline respectively in the two experiments.

In vitro *experiments on cat uterus*

(a) *Effects of adrenaline and noradrenaline in normal solution*

The effects of adrenaline and noradrenaline, tested *in vitro* by the sucrose gap method, differed in three respects from those observed *in vivo*.

First, the non-pregnant uterus was usually less sensitive to adrenaline than pregnant uterus. This is the reverse of the *in vivo* observations.

Second, the ratio of potency of adrenaline and noradrenaline which in virgin cat uterus *in vivo* was between 20 and 100, was only about 10 or less *in vitro*.

Third, the biphasic effect of adrenaline seen *in vivo*, both in ovulating and in one pregnant cat, was not seen *in vitro* when adrenaline was purely stimulant and equiactive with noradrenaline.

Figure 9 shows examples taken from two experiments. In the virgin cat uterus continuous spontaneous electrical activity was recorded with very feeble tension

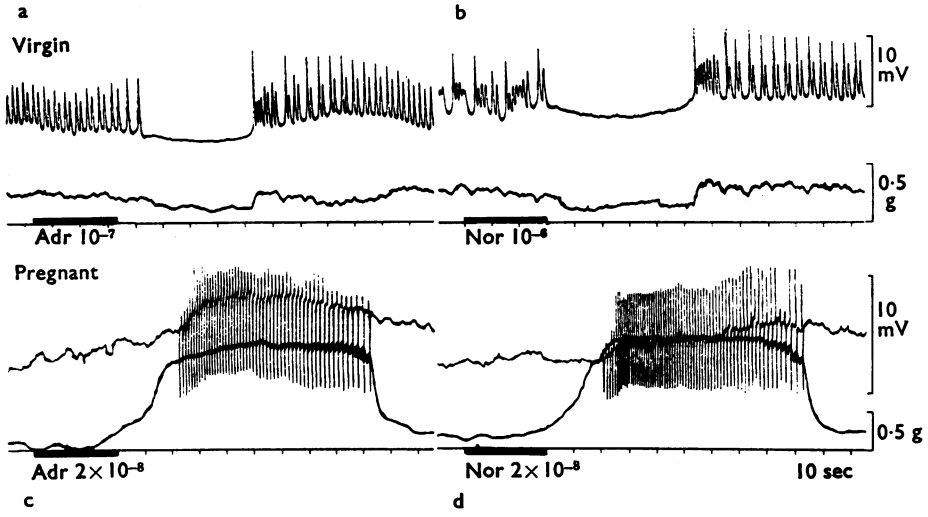

FIG. 9. Cat uterus. Upper trace external (sucrose gap) record of electrical activity, lower trace tension. Relaxation of virgin uterus by (a) adrenaline 10^{-7} g/ml. and (b) noradrenaline 10^{-6} g/ml. Contraction of pregnant uterus by (c) adrenaline 2×10^{-8} g/ml. and (d) noradrenaline 2×10^{-8} g/ml.

development. Adrenaline, and also noradrenaline in a concentration 10 times higher, caused inhibition. In the pregnant cat uterus spontaneous activity was absent or very rare. Both amines evoked a burst of spikes associated with an increase in tension. Their potency was usually the same. They produced stimulation both in the resting muscle and also in spontaneously active muscle.

(b) *Effects of changing the external calcium concentration*

In many uterine strips, particularly of virgin uterus, it was difficult to obtain records with the sucrose gap method. Moreover, the preparations quickly deteriorated and for this reason the observations on the effects of changing the external ion concentration were made on only a few preparations. Clear effects were observed with changes of the external calcium concentration on uterine activity and on the action of adrenaline. In all instances the actions of adrenaline and noradrenaline were affected in the same way.

The virgin cat uterus was very sensitive to changes in the external calcium concentration. Reduction from the normal concentration (2.5 mM) to 0.5 mM briefly increased and then, within a few minutes, abolished spontaneous activity. Excess calcium (7.5 mM) immediately increased the frequency of the spontaneous discharge and increased the tone (Fig. 10). The inhibitory effect of adrenaline was reduced in high calcium and potentiated when the calcium concentration was reduced again (Fig. 10a, b and c). Because the membrane potential and the level of spontaneous activity changed with the changes of the external calcium concentration, however, the effects produced by adrenaline were not strictly comparable.

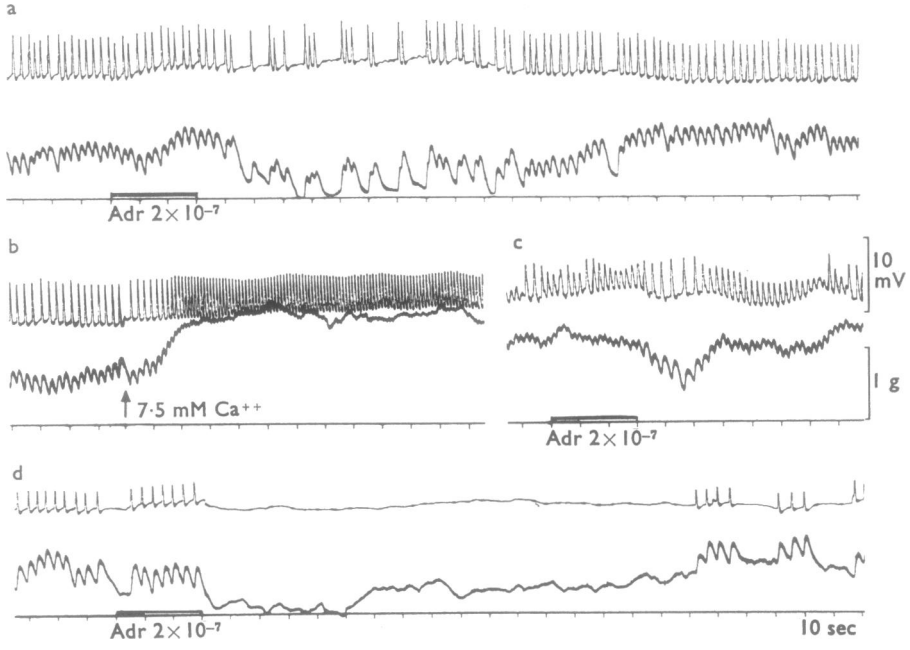

FIG. 10. Virgin cat uterus. (Records as in Fig. 9.) The effect of excess Ca. (a) Effect of adrenaline 2×10^{-7} g/ml. in normal solution, 2.5 mM Ca ; (b) effect of raising the external Ca to 7.5 mM ; (c) effect of adrenaline 15 min later ; (d) effect of adrenaline 35 min after reducing the Ca concentration to 2.5 mM.

In the early pregnant cat uterus low calcium (0.5 mM) soon abolished the stimulant effects of adrenaline. A threefold increase of external calcium caused itself, in the quiescent preparation, during the first few minutes spontaneous bursts of discharge. If spontaneous bursts had occurred before, these became transiently more frequent. The effect of adrenaline was however not changed in the presence of 7.5 mM Ca. A further increase to 12.5 mM increased the action of adrenaline at first (Fig. 11, a and b), but during prolonged exposure the effect became smaller (Fig. 11c). Returning to the normal calcium concentration depressed the effect of adrenaline (Fig. 11d), in the same way as a calcium deficient solution.

In vitro *experiments on guinea-pig uterus*

The difficulties in recording with the sucrose-gap method also limited the number of these observations.

(a) *Effects of adrenaline and noradrenaline*

The guinea-pig uterus showed, in all conditions, spontaneous bursts of activity, separated by intervals of 10 sec to several min. In preparations in which the rhythm was regular and the intervals were sufficiently long, adrenaline and noradrenaline could be tested during the quiescent period. In this condition both caused a series of bursts of spikes and contractions as shown in Fig. 12a. They were equiactive.

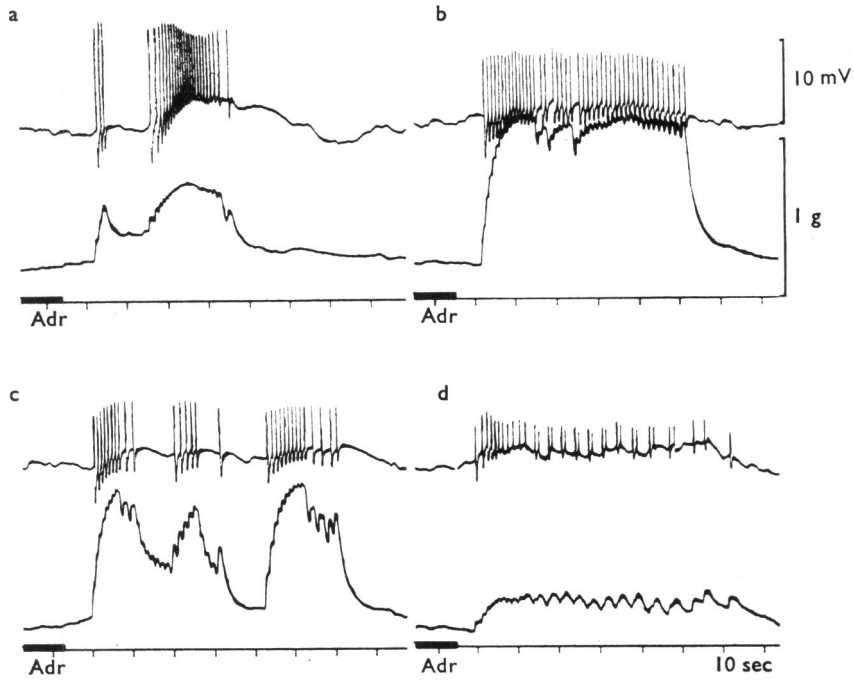

FIG. 11. Pregnant cat uterus. (Records as in Fig. 9.) Effect of adrenaline 2×10^{-7} g/ml. (a) in normal solution (2.5 mM Ca); (b) 15 min after raising the Ca concentration to 12.5 mM; (c) 10 min later; (d) 15 min after reducing the Ca concentration to normal 2.5 mM.

If applied during spontaneous activity, the effects were biphasic. With adrenaline, the inhibitory phase and with noradrenaline the excitatory phase dominated. No consistent reversal could be demonstrated during pregnancy, nor as a result of treatment with oestradiol and progesterone.

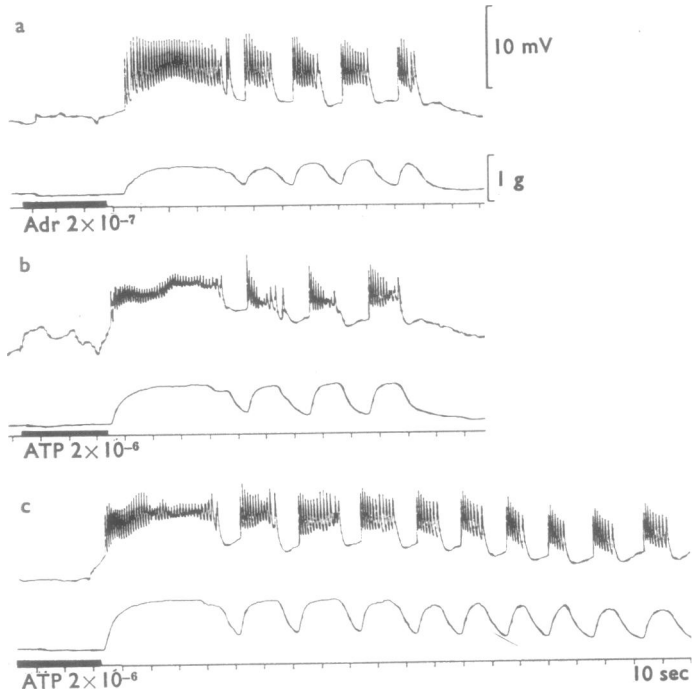

FIG. 12. Non-pregnant guinea-pig uterus. (Records as in Fig. 9.) Effect of (a) adrenaline 2×10^{-7} g/ml. and of (b) ATP 2×10^{-6}M in normal solution; (c) ATP in the presence of 12.5 mM Ca.

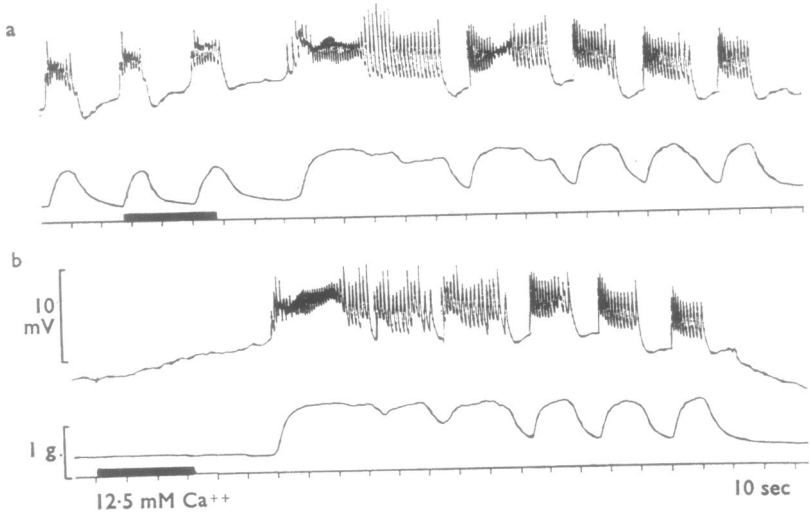

FIG. 13. Non-pregnant guinea-pig uterus. (Records as in Fig. 9.) Effect of raising the external Ca concentration to 12.5 mM for 30 sec (a) during spontaneous activity, (b) during a silent interval.

(b) *Effects of calcium*

The effects of changing the external calcium concentration were the same in the virgin and pregnant guinea-pig uterus, and also after treatment with oestradiol and progesterone. A reduction of the external calcium concentration (0.5 mM) diminished the inhibitory as well as the excitatory effects of adrenaline and noradrenaline.

Excess calcium (7.5 mM and 12.5 mM) had a strong excitatory action itself. Figure 13 shows the effect of exposure for 30 sec to high calcium during (a) a phase of spontaneous activity, and (b) during a quiescent phase. Bursts of activity occurred at shorter intervals, they were more prolonged, and the spikes were discharged at a higher frequency. When adrenaline or noradrenaline were applied during the high activity in the presence of excess calcium, they caused a greater inhibition than in normal solution. If they were administered during a quiescent period, their stimulant effect was also increased in the presence of excess calcium.

Effect of adenosine triphosphate

It is known that the effects of energy rich phosphate compounds on smooth muscle resemble those of the catecholamines (Drury, 1936 ; Fujita, 1954 ; Bueding, Bülbring, Gercken, Hawkins & Kuriyama, 1967). In the present experiments low concentrations of adenosine triphosphate (ATP ; 10^{-6}M to 2×10^{-5}M) caused inhibition in the virgin cat uterus and excitation in the early pregnant cat uterus. Equiactive concentrations of catecholamines were about 100 times lower.

In the guinea-pig uterus, when given during a quiescent period, ATP caused a burst of spikes and contraction like adrenaline and noradrenaline (Fig. 12b). When given during spontaneous activity the effect of ATP resembled that of noradrenaline more than that of adrenaline, for the stimulant component of its diphasic effect was more pronounced.

In both species, the stimulant effect of ATP was, like that of adrenaline, greatly influenced by the external calcium concentration, being abolished by calcium deficiency and enhanced by calcium excess (Fig. 12c).

Discussion

In all animal species so far investigated the membrane potential of uterine muscle cells increases by about 20 mV during pregnancy (Thiersch, Landa & West, 1959 ; Kuriyama, 1961 ; Kuriyama & Csapo, 1961 ; Marshall, 1962). This considerable rise occurs without a change in the intracellular potassium concentration. From the results obtained on rat uterus exposed to different external potassium concentrations, Casteels & Kuriyama (1965) concluded that, during pregnancy, there was chiefly an increase in potassium permeability and, possibly, also a decrease in sodium permeability.

The present experiments indicate that also in guinea-pig and cat uterus changes in membrane permeability occur during pregnancy. Cat uterus, however, differs from rat and guinea-pig uterus in several ways.

(1) Spontaneous activity is rare or absent in early pregnant cat uterus while the uterus of rat and guinea-pig is spontaneously active throughout pregnancy.

(2) The shape of the action potential recorded from early pregnant cat uterus is strikingly different not only from that in the virgin cat, but also different from that in the uterus of rat and guinea-pig under any conditions. The large after-hyperpolarization, the size of which depends on the external potassium concentration, indicates a movement of the membrane potential towards the potassium equilibrium potential. When the external chloride concentration is reduced to 34 mM, the membrane is depolarized, but the absolute value of the after-hyperpolarization remains unchanged.

(3) Excess calcium causes no hyperpolarization in the early pregnant cat uterus, although it raises the membrane potential in the virgin uterus.

(4) In the cat, a change occurs in the chloride distribution during pregnancy. In the virgin cat uterus the chloride content is as low as in rat uterus, but in early pregnancy it is nearly doubled. As a result, the calculated chloride equilibrium potential shifts from -25 mV in the virgin to -11 mV in the early pregnant cat uterus. The large discrepancy between the membrane potential and the chloride equilibrium potential indicates that the chloride in the myometrium is not passively distributed. A similar non-passive distribution has been described in the taenia coli by Casteels & Kuriyama (1966) and there may be an active transport of chloride into the cell against its electrochemical gradient (Casteels, 1965, a, b).

It is tempting to speculate that the change in the intracellular chloride content in cat myometrium during pregnancy is related to the reversal of the response of the tissue to adrenaline. It has recently been shown that noradrenaline (Jenkinson & Morton, 1967) and adrenaline (Bülbring, Goodford & Setekleiv, 1967) increase the K permeability in guinea-pig taenia coli. Bülbring & Tomita (1968) demonstrated that not only the K conductance but also the Cl conductance was increased. If adrenaline had a similar action on the myometrium, this could explain the reversal of its action during pregnancy ; the increase in K permeability might be predominant in virgin uterus, but the increase in Cl permeability might be predominant in pregnant uterus. The Cl equilibrium potential is here considerably less than the membrane potential and, as pointed out by Keynes (1963), in this situation an increase in Cl conductance would have a depolarizing and excitatory effect. Adrenaline has been shown to increase the inward movement of chloride ions in frog skin (Koefoed-Johnson, Levi & Ussing, 1952), but such an action has not yet been demonstrated in other tissues. Further investigation of this aspect is in progress.

It is possible that the inhibitory as well as the excitatory action of adrenaline are primarily due to an increased metabolic energy supply to the cell membrane (Bülbring, Bueding, Gercken, Hawkins & Kuriyama, 1967). Both actions are mimicked by the administration of low concentrations of ATP. Whether the effect of adrenaline is inhibitory or excitatory would then depend on the ratio of its effects on membrane permeabilities to different ions and the emphasis of its action may shift during the course of pregnancy from influencing the distribution of one ion to that of another.

This work was supported, in part, by the U.S. Public Health Service (Grant No. GM 10404) and, in part, by the Medical Research Council (Grant No. G964/340/B).

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(*Received June* 10, 1968)