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EFFECTS OF IONS AND DRUGS ON CELL MEMBRANE ACTIVITY AND TENSION IN THE POSTPARTUM RAT MYOMETRIUM

BY I. A. CSAPO AND H. A. KURIYAMA*
From the Rockefeller Institute for Medical Research, N.Y., U.S.A.

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The electrical properties of uterine smooth muscle have been studied by many authors. However, the measurements obtained vary widely, not only because it is difficult to obtain quantitative data in cells of small size, but also because of changes in the endocrine control of the uterus during the oestrous cycle, gestation and puerperium. For example, (i) during gestation, the membrane potential was shown to increase gradually until just before delivery, when it fell slightly; after delivery the membrane potential dropped rapidly and remained low until the next gestation (Goto & Csapo, 1959); (ii) the placental site of the uterus always showed a higher membrane potential than the non-placental site during all except the latest stage of gestation (Goto & Csapo, 1959; Thiersch, Landa & West, 1959; Kuriyama, 1961*a*, *b*); (iii) *in vivo* treatment with ovarian hormones induced a higher membrane potential (Marshall, 1959; Goto & Csapo, 1959; Jung, 1960) than the highest recorded during gestation (Goto & Csapo, 1959; Kuriyama & Csapo, 1961*a*; Kuriyama, 1961*a*); (iv) the rabbit uterus on the 25th day of gestation still had no spontaneous activity, not even after treatment with high doses of oxytocin (50 m-u./ml.). However, after several hours of storage in oxygenated Krebs's solution the membrane activity was triggered by treatment with oxytocin (1 m-u./ml.) and after storage for more than 24 hr the tissue sometimes showed spontaneous membrane activity (Kuriyama & Csapo, 1959*b*; Csapo, 1961).

In order to rule out effects induced by changes in hormonal status, the rat uterus has been used in the present experiments as a standard preparation from 3 to 6 hr after delivery. The aim of the experiments was to measure the membrane and mechanical activity in relation to changes in the ionic environment.

The results show that in response to various ionic changes the membrane potential and the activity of the postpartum rat myometrium is fundamentally similar to another well studied smooth muscle, the taenia coli

* Present address: Department of Pharmacology, University of Oxford.

(Holman, 1958; Bülbring & Kuriyama, 1963*a*; Kuriyama, 1963). The quantitative differences which have been observed in these two tissues will be discussed in relation to endocrine regulation. Part of this work has been reported by Kuriyama (1961*b*).

METHODS

The experiments were carried out on uterine strips of Sprague-Dawley white rats 3–6 hr post partum. Under ether anaesthesia the uterus was excised and placed in oxygenated mammalian Krebs's solution at room temperature. The composition of the Krebs's solution was (mM): NaCl 118·5, $NaHCO_3$ 24·9, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2; and glucose 1 g/l. It was oxygenated with 95 % O_2 and 5 % CO_2.

The uterus was dissected into a strip 3 mm wide and 30 mm long (at *in vivo* lengths) for measuring the membrane activity. A glass capillary electrode with a resistance of 20–50 MΩ, was mounted flexibly by the method of Woodbury & Brady (1956). The insertion of the electrode into a cell was controlled by a micromanipulator. The tension was recorded simultaneously with the membrane activity by means of a Grass strain gauge (FT02). The membrane and action potentials and the deflexion of the gauge were amplified and recorded by a cathode-ray oscilloscope (Tektronix Model 502).

For the recording of tension in the myometrium we used the fluorescent-dye method (Mashima & Csapo, 1957; Kuriyama & Csapo, 1961*b*). Uterine strips 5 cm long and 3 mm wide were used. The uterine strip was marked off into four segments by spotting on its surface a non-toxic fluorescent dye (RCA material No. 33-Z-607). After the muscle had been loaded with 5 g it was stimulated and the movement of the illuminated marks was photographed on film by a constant-speed motion picture camera (Dumont, Type 321-A). The intensity of the 60 c/s a.c. stimulus was varied while the duration of stimulation was fixed at 5 sec. The muscle was stimulated by ring-shaped platinum wire electrodes, either at one end of the strip with two electrodes placed close together or else with one electrode at each end of the strip.

RESULTS

Effect of ions on the membrane potential

The mean value of the membrane potential has been given previously (Kuriyama & Csapo, 1961*a*), i.e. 48 mV ± 0·48 (standard error of mean, $n = 130$) in the parturient and the postpartum rat uterus. This value is 4 mV lower than that of the 18–20th day pregnant rat uterus (52 mV ± 0·61 ($n = 53$)) and is 8 mV higher than that of the rat uterus 24 hr post partum (40 mV ± 0·68 ($n = 38$)).

K ion. A reduction of the potassium concentration to 3 mM increased the mean membrane potential from 52 to 57 mV. Higher concentrations of potassium decreased the membrane potential, e.g. ten times the normal potassium concentration (60 mM) depolarized the membrane to 23 mV and twenty times (120 mM) depolarized the membrane to 13 mV but not to zero.

The relationship between the membrane potential and log of external K concentration in the presence of chloride and sulphate is illustrated in Fig. 1. The maximum slope of the variation in the membrane potential of the postpartum rat uterus with tenfold change of $[K_o]$ was 31 mV in the

presence of chloride and 37 mV in the presence of sulphate. The corresponding figure for the pregnant uterus is 35 mV in the presence of chloride. At moderate [K_o] (24 to 3 mM), the slope of the relationship between membrane potential and log [K_o] became less steep (Fig. 1).

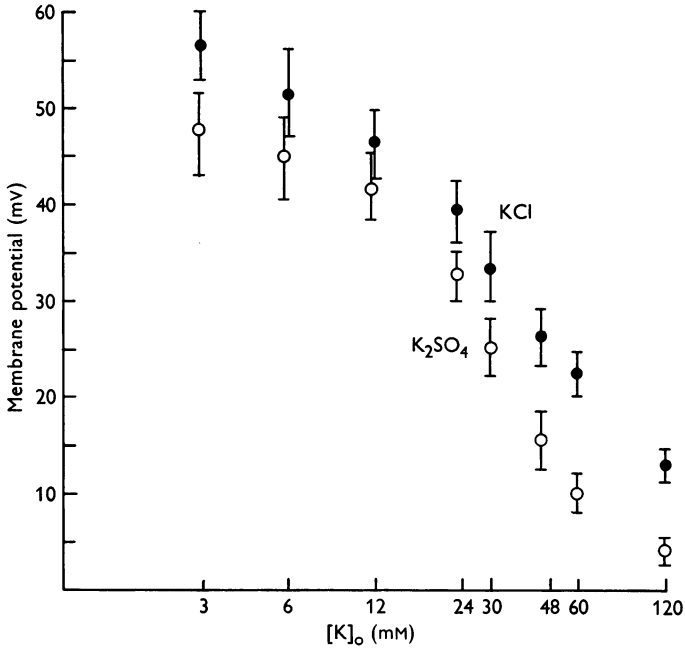

Fig. 1. The relation between membrane potential and the logarithm of the external potassium concentration in the rat myometrium 6 hr post partum, in the presence of chloride and sulphate ions.

Anions. The replacement of chloride by nitrate, iodide or sulphate ions transiently lowered the membrane potential which then gradually returned to a new level. The degree of initial depolarization was higher in sulphate solution (mean value, 12 mV) than in nitrate and iodide (mean values 6 and 8 mV, respectively). Replacement of chloride with nitrate or iodide always caused a subsequent hyperpolarization for a period of 60–90 min (control mean value 49 mV, nitrate 55 mV and iodide 53 mV, respectively), but when the chloride was replaced with sulphate the membrane remained depolarized (mean depolarized value, 43 mV).

Na ion. Excess sodium (Krebs's solution with 104·6 m-mole additional NaCl/l., i.e. 248 mM-Na in all) decreased the mean membrane potential from 49 to 42 mV after 15 min of perfusion. Sodium deficiency (64 mM-Na), with choline or sucrose as substitutes, transiently increased the membrane potential, then gradually decreased it. In a solution in which all the NaCl

was replaced either by sucrose or by choline chloride, the mean membrane potential increased after 30 min perfusion from 48 to 56 and 55 mV, respectively, and decreased after 90 min of exposure to 50 and 41 mV, respectively. After 180 min of exposure mean membrane potentials decreased to 45 and 39 mV, respectively. The degree of depolarization was more marked in the sucrose solution than in the choline solution.

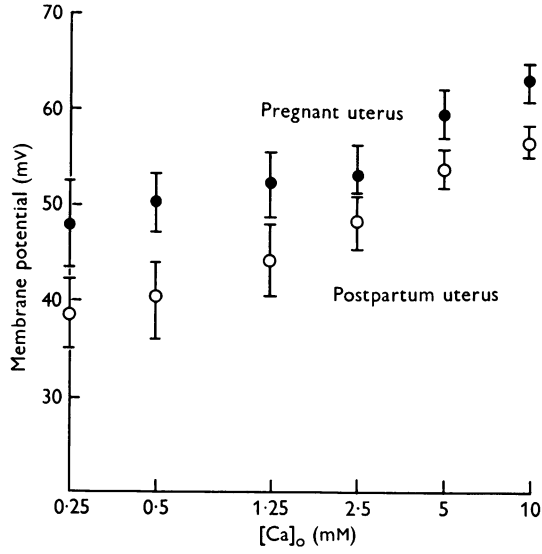

Fig. 2. The relation between membrane potential and the logarithm of the external calcium concentration in the pregnant (18th and 19th days) and postpartum myometrium (6 hr.)

Ca ion. When the external calcium concentration was decreased from normal (2·5 mM) to one tenth, the membrane potential decreased, after 30 min of exposure, from 48 to 39 mV. Excess calcium (7·5 mM) increased the membrane potential, after 30 min of exposure, from 48 to 54 mV. Figure 2 shows the effect of various Ca concentrations on the membrane potential of the postpartum myometrium. For comparison, the effect of calcium on the pregnant myometrium is also illustrated. A difference was found in that calcium deficiency decreased the membrane potential more slowly in the pregnant uterus than in the postpartum myometrium. This observation is in good agreement with that of Goto & Csapo (1959) concerning the effect of calcium deficiency on the pregnant and postpartum rabbit uterus. As is shown in Fig. 2, in excess calcium the membrane potential increased more in the pregnant than in the postpartum myometrium. The membrane potential of the pregnant uterus in normal Krebs's solution is also higher than that of the postpartum uterus.

Effect of changes in the ionic environment on the membrane activity and contraction of the rat myometrium

Difficulties in the quantitative analysis of the electrical activity of the myometrium

Kuriyama & Csapo (1961*a*) observed that the postpartum myometrium usually shows more regular train discharges than the pregnant myometrium (amplitude of spike is 44 mV $\pm$ 0·5 (n = 115), half-duration of the spike is 47 msec $\pm$ 3·1 (n = 30)). The number of spikes in a train was 44 $\pm$ 3·1 (n = 45) and the frequency of spikes was 0·73 $\pm$ 0·02/sec (n = 86). The typical regular membrane activity and tension of the postpartum myometrium are illustrated in Fig. 3. The number of spikes in a train, the spike amplitude, the pause between the trains and the shape of tension development were almost constant and the membrane potential was stable.

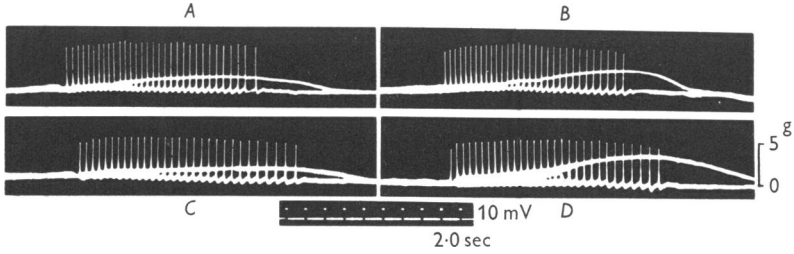

Fig. 3. Simultaneous recording of the membrane activity (from a single cell) and of the tension in the rat myometrium, 6 hr post partum. Upper (*A* and *B*) and lower (*C* and *D*) traces are recorded from the same cell.

In some specimens irregularities occurred owing to heterogenic pacemaker activity and these trains are illustrated in Fig. 4. It can be seen that there is either a slowing or an acceleration of the frequency of the spikes. Heterogenic excitation may also induce a slight depolarization with increased frequency, or a slight hyperpolarization with decreased frequency. Changes in the membrane potential and in the frequency of the spikes are always related to changes in tension. Heterogenic excitation complicates the study of the effects of ions on the electrical activity of the uterus.

Another experimental difficulty arises from differences between individual cells. It is very difficult to keep an inserted micro-electrode in a single cell for a long period. Therefore the records have to be obtained, before and after treatment of the tissue, from different cells. Figure 5 shows records from three cells in the same tissue, in which the shape of tension development is almost the same. However, their membrane potentials are 58, 54 and 51 mV, and the action potentials are 50, 46 and

44 mV, respectively. The number and frequency of the spikes also differ considerably. The cell having the highest membrane potential blocked spike generation to a great extent and only retained electrotonic potentials at certain times. This variation adds to the difficulty of making quantitative observations of the action of ions and drugs on the myometrium.

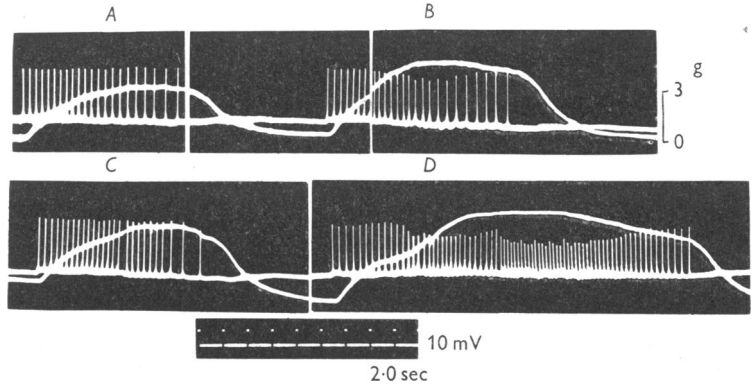

Fig. 4. Rat myometrium 6 hr post partum. Simultaneous recording of action potentials from a single cell and of the tension. The spike frequency, number and amplitude vary in the individual train discharges *A*, *B*, *C* and *D* (and also tension developments) although the micro-electrode is impaled in the same cell.

Effect of changes in the ionic environment on the membrane activity and contraction

K ion. Increased potassium concentration accelerated membrane activity. In 3 times the normal potassium concentration the mean values of the maximum rates of rise and fall were decreased from 9·6 V/sec to 6·7 V/sec and from 6·2 V/sec to 4·8 V/sec, respectively. The spike amplitude decreased from 46 to 42 mV but the frequency of the spikes increased from 0·86/sec to 1·46/sec. After 5–10 min exposure in 5 times the normal $[K_o]$ the rates of rise and fall decreased to 3·4 V/sec and 3·1 V/sec, respectively and the spike amplitude decreased to 37 mV. The frequency of the spike did not increase more than in 3 times the normal $[K]_o$. Spike generation is often blocked after 15–30 min of exposure in 5 times normal $[K]_o$ and often contracture of the muscle is observed accompanied by small periodic potential changes without spike generation. Figure 6 shows the effect of various $[K]_o$ on membrane activity and tension. Figure 7 shows the effect of various $[K]_o$ on the generation and propagation of contraction in the postpartum myometrium. In these experiments the fluorescent dye method was used. This method was useful in observing the pattern of contraction in various parts of a tissue, and hence the location of the pacemaker region and the pathway and velocity of the contraction. Figure 7*A*

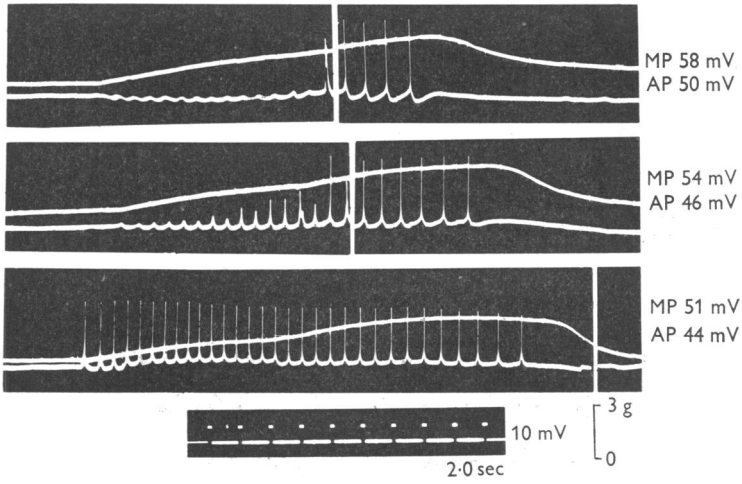

Fig. 5. Rat myometrium 6 hr post partum. Simultaneous recording of action potentials from a single cell and of the tension. Upper, middle and lower traces are recorded from different cells in the same tissue. The spike amplitude and number in a train depend on the membrane potential in each individual cell. However, the shape and force of the tension are almost the same.

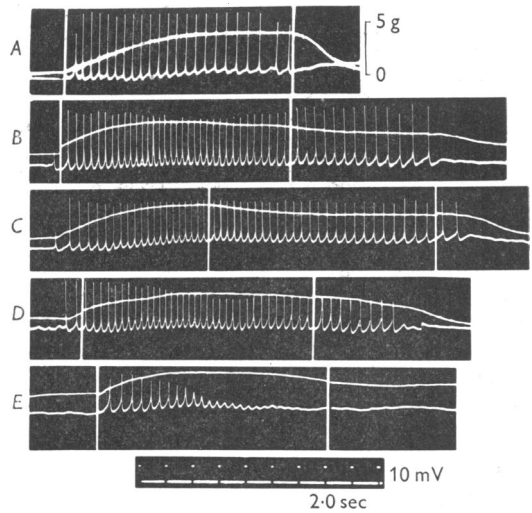

Fig. 6. Effects of various concentrations of potassium chloride on the membrane activity and tension in the postpartum rat myometrium. *A*, normal, 6 mM-KCl. *B*, 12 mM-KCl. *C*, 18 mM-KCl. *D*, 24 mM-KCl. *E*, 30 mM-KCl. 30 mM-KCl causes block of the spike discharges and induces contracture.

shows the effects of stimulating a strip at both ends (*A* 1) and at one end (*A* 2). At *A* 1 there is a short latency of the contraction in whole tissue. At *A* 2 the delay of contraction in the non-stimulated end is marked and is due to the time required for propagation of the excitation. Conduction velocity of the contraction is 9·6 cm/sec ± 0·3 (n = 10). This value is nearly the same as that determined for spike propagation in the pregnant mouse myometrium (mean value 10·6 cm/sec; Goto, Kuriyama & Abe, 1961). In Fig. 7*B* a strip of myometrium is stimulated at both ends by

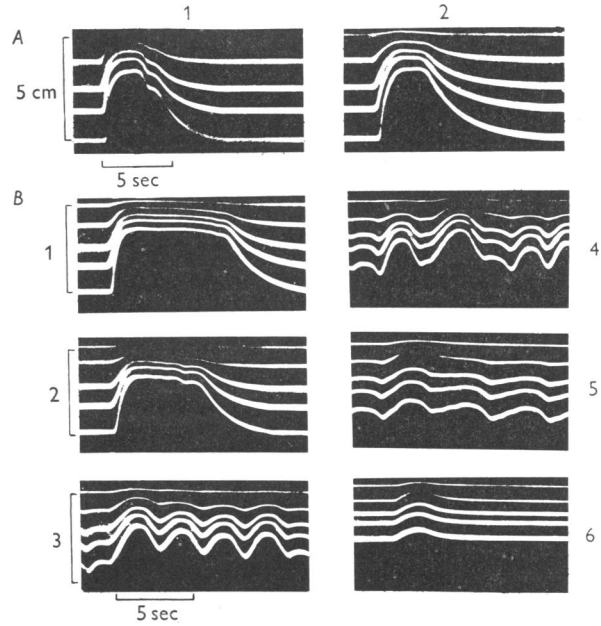

Fig. 7. Effects of various concentrations of potassium chloride on the contraction of the postpartum rat myometrium, by the fluorescent dye method. *A* shows the effects of stimulation of a strip at both ends (1) and at one end (2). *B*. The potassium concentrations were increased in steps from 3 mM (1) to 6 mM (2), 18 mM (3), 24 mM (4), 30 mM (5) and 42 mM (6), while the strip was stimulated at both ends by alternating current (see text).

alternating current (1·5 V/sec intensity, 5 sec duration). The potassium concentration was increased in steps from 3 to 42 mM. In twice the normal potassium concentration the spontaneous contractions appeared at the relatively low bath temperature of 30° C. Concentrations of potassium 3–5 times normal increased the amplitude and frequencies of the spontaneous contractions, while in a concentration of 7 times normal the spontaneous activity ceased and the muscle was shortened to 50 % of its original length. However, electrical stimulation still induced a contracture.

Nitrate ion. Nitrate increased membrane activity and tension. The spike number and the frequency of the train discharge were also increased. When the muscle was kept for 15 min in nitrate solution, the spike frequency increased from 0·9/sec to 1·6/sec. The spike amplitude decreased slightly from 49 to 44 mV and the rates of rise and fall changed from 10·6 V/sec to 9·2 V/sec and 8·4 V/sec to 4·3 V/sec, respectively. After 30 min in nitrate solution, the spike frequency was still high (1·3/sec).

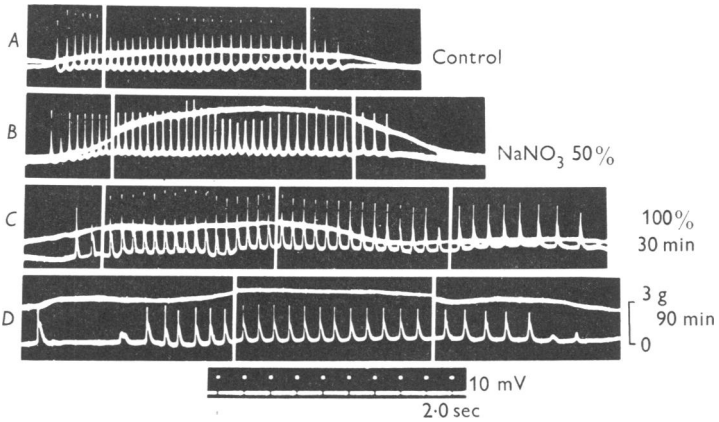

Fig. 8. Effects on the postpartum rat myometrium of replacing chloride with nitrate. *A*, control. *B*, 50% of NaCl replaced by $NaNO_3$ (after 30 min). *C*, NaCl is completely replaced with $NaNO_3$ (after 30 min). *D*, after 90 min the repolarization phase of the spikes is prolonged by treatment with nitrate.

The spike height remained unchanged (48 mV) but the rates of rise and fall were decreased (5·8 V/sec and 2·8 V/sec, respectively). The most typical change of membrane activity appeared to be a prolongation of the spike duration. The repolarization phase of the spike formed a plateau. The half-duration of the spike was prolonged from 47 to 520 msec when the muscle was kept for 30 min in nitrate solution. After prolonged exposure to nitrate the spike frequency in the train decreased, but the pause between two train discharges became shorter and shorter until subsequent trains fused. The spike amplitude decreased only after 1–2 hr. Tension did not decrease although the spike frequency was decreased. Figure 8 shows the effect of the replacement of chloride by nitrate on the membrane activity and tension.

Iodide ion. Iodide also increased the membrane activity and tension. The spike amplitude did not decrease for at least 30 min. During long exposure (after 60 min) the amplitude decreased from 51 to 42 mV and the duration of spike was prolonged from 47 to 81 msec. The frequency of the spike discharge increased from 0·78/sec to 1·2/sec after 30 min exposure while it decreased to 0·86/sec after 60 min exposure.

Sulphate ion. Treatment with sulphate ion caused an initial acceleration of membrane activity and tension followed by depression. At the stage of depression, after 30 min, the membrane was depolarized by 6 mV and the amplitude of the spike was decreased from 49 to 41 mV. The spike frequency decreased from 1·0/sec to 0·6/sec. The effect of sulphate on the contraction was observed by the fluorescent dye method. Sulphate ion decreased tension development. This effect was more marked than the delay of the propagation of contraction.

Na ion: deficiency. When the sodium chloride of the Krebs's solution was replaced with choline chloride or with isotonic sucrose (24 mM of sodium bicarbonate remaining in solution), the membrane activity and tension development were increased. After 30 min the spike amplitude was slightly decreased from 47 to 44 mV, the rates of rise and fall were decreased from 7·9 V/sec to 4·1 V/sec and from 6·1 V/sec to 3·0 V/sec, respectively, while the spike frequency increased from 0·81/sec to 1·2/sec. Even 2–3 hr after replacement with choline the membrane activity remained high, but it did not in sucrose solution in which, after 1–2 hr, the membrane activity decreased. In choline chloride solution after 2 hr exposure the spike amplitude decreased to 38 mV, the rates of rise and fall decreased to 3·8 V/sec and to 2·1 V/sec, respectively, and the spike frequency remained high (1·1/sec). In sucrose solution after 2 hr exposure the spike amplitude decreased to 31 mV, the rates of rise and fall decreased from 1·3 V/sec to 0·8 V/sec and the spike frequency decreased to 0·46/sec. Figure 9 shows the effect (after 9 and 180 min of exposure) of the replacement of sodium chloride by choline chloride. The differences between the changes produced by choline chloride and sucrose were not confined to membrane activity but extended to the processes responsible for development of tension. Dissociation of the tension development from spike activity (i.e. gradual decrease of tension while spike discharge was maintained) appeared in sucrose solution but not in choline chloride.

Na ion: excess. 248 mM-Na decreased the membrane potential but increased the spike frequency and the spike amplitude. The overshoot potential increased to 20 mV. However, this augmentation was not maintained. The shape of the spike deteriorated, the rates of rise and fall gradually decreased. The maximum rates of rise and fall in normal Krebs's solution were 10·5 V/sec and 6·3 V/sec, respectively. After 15 min exposure in excess sodium these values increased to 14·2 V/sec and 8·4 V/sec, respectively, while after 45 min they decreased to 6·7 V/sec and 4·8 V/sec, respectively. The spike frequency increased after 15 min from 0·72/sec to 1·1/sec, while after 45 min exposure it decreased to 0·32/sec.

Ca ion. Excess calcium (5 and 7·5 mM) increased the membrane potential and decreased the spike frequency. In 7·5 mM-Ca the spike amplitude

increased from 53 to 62 mV, the membrane potential increased from 47 to 53 mV, and the maximum rates of rise and fall increased from 8·6 V/sec and 6·5 V/sec to 15·1 V/sec and 11·6 V/sec, respectively. The spike frequency decreased from 1·0/sec to 0·4/sec. In high Ca concentrations the membrane activity often increased only transiently, then gradually decreased.

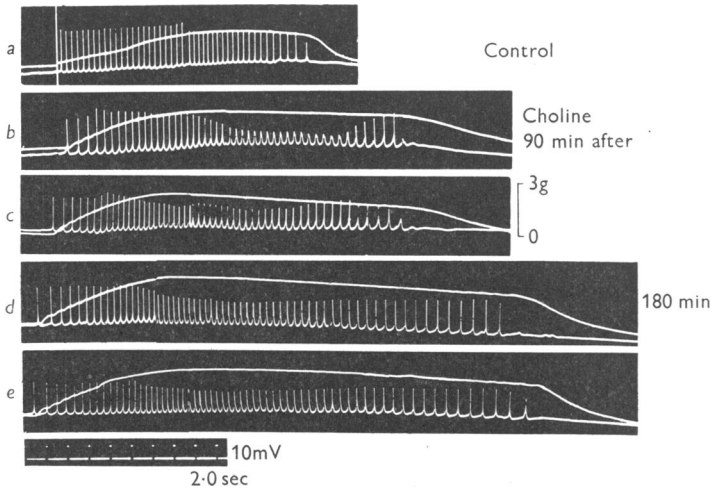

Fig. 9. Effect of choline chloride replacing sodium chloride on the postpartum rat uterus. *a*, Control. *b* and *c* are successive records 90 min after replacement with choline chloride, *d* and *e* after 180 min. Continuous lines are tension records.

In Ca deficiency the membrane was depolarized and the spike frequency increased. Gradually the membrane activity decreased. For example, when the calcium concentration was decreased to one tenth of the normal concentration (0·25 mM) the membrane was depolarized, and after 30 min only small spikes could be recorded.

Effects of acetylcholine and adrenaline on membrane activity and tension in the postpartum rat myometrium

Acetylcholine. Treatment with acetylcholine 10^{-6} M (as the chloride) depolarized the membrane by about 3–17 mV, giving values of 32–70 mV (mean 48). The size of the action potential was decreased by 5–10 mV (mean 44 mV), while the half-duration of the spike was slightly prolonged or remained unchanged. The number of spike discharges was increased. The trains appeared more frequently and contained a larger number of spikes. About 15 min after the application of acetylcholine the membrane activity returned to normal. In a few experiments the membrane potential was not changed, but there was a sudden increase in spike frequency which

resulted in depolarization. Eventually the membrane potential and the frequency returned to the original levels. The duration of the effect of acetylcholine was proportional to the concentration.

In one experiment (Fig. 10) the trains discharged by the untreated uterus had only a few spikes, which were not well synchronized with the contraction (*a*); the membrane potential was rather higher (58 mV) than the average. Treatment with acetylcholine 10^{-6} M decreased the membrane potential and increased the number of spikes in the train (*b*). After 10 min the discharges became regular and remained so; the membrane potential was now 52 mV. This suggests that cells of low excitability were activated in the presence of acetylcholine, enforcing synchrony in the tissue.

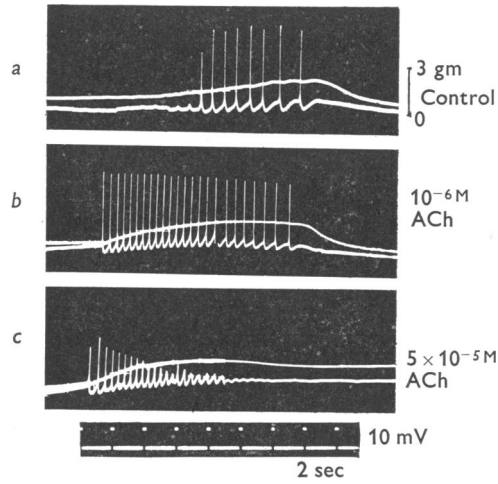

Fig. 10. Effect of acetylcholine on membrane activity and tension. *a*, Control. *b*, 10^{-6} M ACh. *c*, 5×10^{-5} M ACh. *a* and *b* are recorded from the same cell; see text. 5×10^{-5} M ACh induced depolarization block of membrane activity, and contracture occurred without spike discharge.

A higher concentration of acetylcholine ($> 5 \times 10^{-5}$ M) caused contracture of the muscle (*c*). The membrane was depolarized by 10–20 mV and the spike frequency first increased, then deteriorated to oscillations. However, the contraction continued for a long period, showing some fluctuation but no relaxation. It appears therefore that at certain levels of depolarization acetylcholine produces a contracture.

Acetylcholine increased the force and frequency of contractions. Its effect was proportional to the concentration, and activity returned to control levels after washing out the drug. The degree of tension induced by acetylcholine depended on the initial tension.

Adrenaline 10^{-7} M (as the hydrochloride) induced slight hyperpolarization (5–10 mV) and decreased the number of spikes in the train; it in-

creased the amplitude of the action potentials from 45 to 53 mV in 8 min, prolonged the pre-potential, and slightly raised the threshold for spike generation (by 3–5 mV). Adrenaline 10^{-6} caused a greater degree of hyperpolarization and stopped spike generation. After 20–30 min the activity returned, even in the presence of the same concentration of adrenaline.

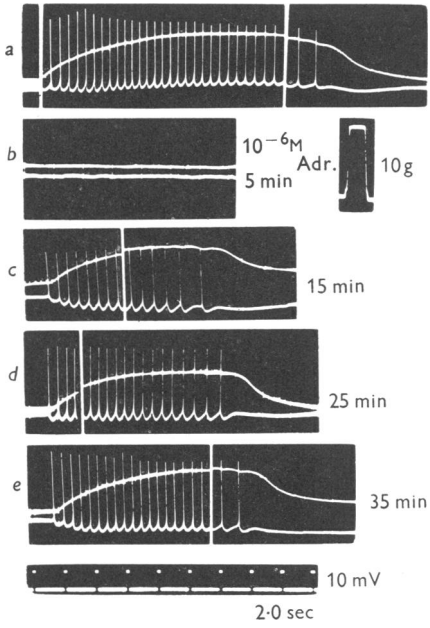

Fig. 11. Effect of adrenaline (10^{-6} M) on the postpartum rat myometrium. *a*, Control. *b*, 5 min after administration of adrenaline: all activity ceased, and the membrane was hyperpolarized. *c*, after 15 min. *d*, after 25 min. *e*, after 35 min.

However, the spikes were few and infrequent. Recovery to control levels was slow (Fig. 11). In some cases the spikes disappeared before hyperpolarization developed. This effect may be compared with that of progesterone (Kuriyama, 1961*a*), when there is slow hyperpolarization and the spikes disappear before the hyperpolarization develops. Adrenaline 10^{-6} also inhibited membrane activity of the uterus at the 15–16th day and at the 19–20th day of gestation.

Low concentrations of adrenaline do not change the frequency of spontaneous contractions in the postpartum myometrium, but reduce the tension. Higher concentrations abolish the contractions suddenly, and the muscle becomes relaxed. After a silent period contractions reappear but their frequency is low, in contrast with the behaviour of the uterus after progesterone treatment.

Adrenaline may affect tension in a different way. A low concentration may decrease the frequency of contraction without changing the force; a higher concentration may decrease the frequency still further and sometimes may stop contraction altogether.

DISCUSSION

The membrane potential of the postpartum myometrium was more sensitive to changes of external potassium concentrations than to other ions. However, in the low range of $[K]_o$ (from 3 to 18 mM) the relation between the membrane potential and the logarithm of $[K]_o$ was markedly different from the theoretical potassium potential predicted from the Nernst equation. When the muscle was soaked in low-K solutions which were also Na-deficient, the slope of the line indicating the relationship between the membrane potential and the logarithm of $[K]_o$ was more steep. In solutions containing excess potassium, the slope was less steep in the presence of chloride than in the presence of sulphate. In the 6 mM $[K]_o$ solution, the substitution of chloride by sulphate depolarized the membrane, whereas nitrate and iodide hyperpolarized it. We have not yet measured the permeability coefficients of ions in this tissue, therefore we cannot explain these results further. However, the data of Daniel & Daniel (1957) and Daniel & Singh (1958) suggest that the intracellular sodium and chloride concentrations are much higher in uterine than in skeletal muscle.

The observations described offer the following explanation for the generation of the membrane potential in the postpartum myometrium. In the physiological range of $[K]_o$ the membrane potential is due mainly to the potassium concentration gradient. However, the Na conductance in the resting membrane is relatively high compared with skeletal muscle.

The amplitude of action potential in a cell did not markedly decrease with sodium deficiency. These observations agree with those for the uterus (Daniel & Singh, 1958), for the taenia coli (Holman, 1958; Bülbring, Kuriyama & Twarog, 1962) and for the stomach (Kolodny & van der Kloot, 1961). In the taenia coli this phenomenon was interpreted as being due to a relatively moderate change in the sodium equilibrium potential due to the rapid decrease of $[Na]_i$ following the change of $[Na]_o$ (Bülbring & Kuriyama, 1963*a*). This explanation was supported by Goodford (1962) measuring the ionic distribution across the membrane in the same tissue. The same interpretation might apply in the case of the myometrium.

The rate of rise of the spike was decreased in sodium-deficient, in calcium-deficient and in excess-potassium solutions. The membrane potential of the tissue was 48 mV, therefore the sodium carrier system might be partially inactivated. Thus further depolarization of the membrane by solutions low in calcium or high in potassium should increase

the inactivation of sodium carrier system. These processes may be responsible for the decreased rate of rise of the spike and the sodium deficiency should decrease the influx of sodium ion during the active state of the membrane. In excess calcium solution the rate of rise of the spike was augmented. In the giant nerve fibre, and in skeletal muscle, excess calcium has the same effect on the ionic conductances as hyperpolarization of the membrane as determined by the voltage clamp method (Hodgkin & Huxley, 1952; cf. Shanes, 1958). The calcium in the myometrium may have a role similar to that assumed for other excitable tissues.

Tension developed with some regularity in the rat myometrium post partum and there was good synchrony between spike and tension. However, in the sucrose solution and in the iodide solution dissociation of spikes from tension appeared. The sucrose solution increased the spike frequency but decreased tension. Such a dissociation was not found with the choline solution. The nitrate solution increased the spike frequency and tension. Increased tension was not only due to increased spike frequency, but also to the increase of tension per spike. These phenomena were also observed in other smooth muscles (Axelsson, 1961; Kao & Gluck, 1961).

Acetylcholine 10^{-8} to 10^{-7} M depolarized the membrane, increased the number and frequency of the spikes in the train, and increased tension. High concentrations of acetylcholine ($> 5 \times 10^{-5}$ M) depolarized the membrane to less than 30 mV, reduced the spike discharge to oscillations and maintained a contracture. Adrenaline abolished the spike and reduced the tension. Abolition of the spike had no close relation to the hyperpolarization of the membrane. Marshall (1959) observed that in the rat myometrium treated with oestrogen or both oestrogen and progesterone acetylcholine increased the spike frequency and the contractions, while adrenaline had the opposite effect. Ozaki (personal communication) observed the effect of acetylcholine on the membrane activity of the pregnant mouse myometrium induced by electrical stimulation. He recorded very little change in the half-duration of the spike (14·7 msec changed to 14 msec). However, in the postpartum myometrium described here a low concentration of acetylcholine, 10^{-7} M, increased the rate of rise of the spike and shortened its half-duration without significant increase of its height. But 10^{-5} M acetylcholine increased the spike frequency and lessened the rate of rise. In the taenia coli Bülbring & Kuriyama (1963*b*) observed the effect of acetylcholine and adrenaline in various ionic environments and they concluded that acetylcholine may increase the non-selective permeability to the ions in the same manner as has been inferred for the end-plate region of skeletal muscle (Katz, 1962). They also concluded that adrenaline may reduce the sodium conductance but increase

the active sodium extrusion and that these phenomena have a causal relationship to production of energy-rich phosphate compounds (Axelsson, Bueding & Bülbring, 1961). These conclusions may apply to the myometrium. It is well established that adrenaline has a different action on uterine activity, depending on the endocrine state of the tissue (in the cat uterus, Kennard, 1937; Bozler, 1940; in the guinea-pig uterus, Bozler, 1940; Greefe & Holtz, 1951; Hermansen, 1961). We did not convince ourselves that the pregnant and postpartum rat myometrium displays a characteristically different effect after treatment with adrenaline.

The above experiments indicate that the rat myometrium is fundamentally similar not only to other smooth muscles (for example, taenia coli) but also to skeletal muscle. The mean membrane potential is about 10 mV lower than that of the taenia coli and 30–40 mV lower than that of skeletal muscle. The low membrane potential might be due to poor fixation of calcium in the membrane (Goto & Csapo, 1959; Csapo & Coutinho, 1960; Marshall & Csapo, 1961; Kuriyama, 1961*a*, *b*). This may result in an increase in the resting sodium conductance and an inactivation of the sodium carrier. These changes might explain the low resting potential and the slow rate of rise of the spike.

SUMMARY

1. Effects of various ions, acetylcholine and adrenaline were studied on the membrane activity and tension in rat myometrium, 3–6 hr after delivery.

2. The maximum change of the curve relating membrane potential and extracellular potassium concentration was 31 mV for a tenfold change in the presence of chloride and 37 mV in the presence of sulphate. The membrane potential at the low range of external potassium concentrations behaved as if it were a potassium equilibrium potential, but the sodium permeability was also important in determining its value.

3. More than 30 mM potassium concentration greatly depolarized the membrane and blocked spike generation. During the resulting contracture no spikes were present.

4. Nitrate and iodide ions (substituted for chloride) increased the membrane potential, while sulphate decreased it. The repolarization phase of the spike was prolonged by nitrate and tension was enhanced.

5. In sodium deficiency the membrane potential increased and after 120 min of exposure the overshoot potential was often maintained but the rate of rise of the spike was lowered gradually.

6. The fluorescent dye method was used for the measurement of the velocity of propagation (mean value 9·6 cm/sec). The pattern of the contraction was also studied.

7. Acetylcholine depolarized the membrane and increased the number of spikes in the train, the spike frequency and the train frequency. Adrenaline had an opposite action on the membrane. During gestation as well as post partum no triggering effect was seen.

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