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ELECTRICAL PROPERTIES OF THE COSTO-UTERINE MUSCLE OF THE GUINEA-PIG

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SUMMARY

1. The spontaneous electrical and mechanical activity of the costo-uterine muscle of the guinea-pig are described.

2. The spontaneous electrical activity, recorded intracellularly, is similar to that observed previously in longitudinal myometrium of rat (Marshall, 1959) and ionic substitution suggests that, though calcium may be the predominant ion carrying the current during the upstroke of the action potential, some influence of sodium cannot be ruled out.

3. During dioestrus, when circulating progesterone levels are high, there is an increase in the resting membrane potential and a decrease in the frequency of electrical and mechanical activity.

4. There is a two-fold decrease in the space constant (λ) during dioestrus. At this time the membrane time constant (τ_m) is also decreased.

5. The diameter and length of the smooth muscle cells are smaller during dioestrus. However, the differences in cell diameter do not explain all of the differences observed in λ at this time and it is suggested that there may be an increase in the resistance to current flow between cells.

6. It is concluded that high circulating progesterone may bring about quiescence of target smooth muscle in two ways: by stabilizing the cell membrane and by restricting the spread of activity.

INTRODUCTION

The uterus of many quadrupeds is attached to the last ribs by a strap-like structure, the ovarian suspensory ligament. A detailed histological examination of this tissue in the guinea-pig by Gabella (1976) revealed that it consists predominantly of strands of smooth muscle orientated in a longitudinal direction, which are continuous with the longitudinal smooth muscle bundles of the myometrium. The location and composition of the structure prompted Gabella (1976) to rename it the costo-uterine muscle and this term is used in this paper.

Strands of smooth muscle fibres from the costo-uterine muscle extend throughout the peritoneal mesenteries supporting the upper regions of the female reproductive tract, including the mesotubarium. It has been suggested that contractions of these

smooth muscles may affect ovum capture or transport (Westman, 1929). The aim of this study was to investigate the electrical and mechanical properties of these smooth muscles and to study how they may change as the consequence of the varying patterns of sex steroids during the oestrous cycle. The effects of pre-treatment with the sex steroids on the passive electrical properties of longitudinal uterine smooth muscle have been studied previously in the rat (Abe, 1971; Kuriyama & Suzuki, 1976) whereas this paper describes these effects on longitudinal reproductive smooth muscle of the guinea-pig.

Many references occur in the literature to changes in uterine smooth muscle cell dimensions in response to variations in circulating sex steroids (Brody & Westman, 1960; Ross & Klebanoff, 1967; Bo, Odor & Rothrock, 1969). However, no quantitative histological data have been presented to substantiate these assertions. Furthermore, no attempt has been made to investigate simultaneously the effects of pre-treatment with steroids on cell dimensions and to see whether or not the changes in electrical properties could be attributable to changes in the dimensions of the smooth muscle cells. Because the smooth muscle fibres of the costo-uterine muscle are remarkably parallel (Gabella, 1976) it was hoped that this tissue might be suitable for studying cell dimensions.

A brief account of these results has been given previously (Parkington & Taylor, 1977).

METHODS

Virgin female guinea-pigs (300–400 g) were used throughout this study. The vagina of each animal was inspected twice daily, at 09.00 and 21.00 h. A smear was taken at each inspection from the time when the vaginal membrane first ruptured until it reformed. Maximum leucocytic invasion of the vaginal smear was considered to be the time of ovulation. The costo-uterine muscle was studied during three stages of the oestrous cycle allocated according to circulating oestrogen and progesterone levels as described by Croix & Franchimont (1979) and Blatchley, Donovan & Ter Haar (1976): (i) proestrus, when plasma oestrogen is high and progesterone low; (ii) oestrus, following ovulation when both oestrogen and progesterone levels are high; (iii) dioestrus, when plasma progesterone is high and oestrogen levels are negligible.

The animals were stunned and bled and both costo-uterine muscles were removed. For electrophysiological studies an entire costo-uterine muscle (about 15 mm long; 0·3–0·5 mm^2 cross-sectional area) was mounted in an organ bath which was fitted with a pair of large plate stimulating electrodes and was similar to that described by Abe & Tomita (1968). About 9 mm of the tissue was placed between the stimulating electrodes while about 6 mm protruded into the recording compartment (see Bywater & Taylor, 1980). The tissue was stimulated using a Grass S88 stimulator via an isolation unit. Tissues were perfused at about 2 ml min^{-1} (in a bath with a volume of 2·5 ml) with physiological solution containing (mM): NaCl, 120; KCl, 5; KH_2PO_4, 1; $CaCl_2$, 2·5; $MgSO_4$, 1·2; $NaHCO_3$, 25; glucose, 11; saturated with 95% O_2 and 5% CO_2. The temperature of the solution in the organ bath was maintained at $37 \pm 0{\cdot}5$ °C.

Transmembrane potentials were recorded using conventional glass micro-electrodes filled with 2·5 M-KCl, with resistances of around 60 MΩ. Signals were fed into a high impedance pre-amplifier, displayed on a dual-beam oscilloscope and stored on magnetic tape. Results used were only from those impalements which had been maintained for at least 10 min. Criteria for an acceptable impalement were: (i) a sharp negative deflexion of the voltage trace from the zero level: (ii) a steady base line; (iii) a spontaneous or induced action potential; (iv) the return of the voltage to zero following withdrawal of the electrode.

Spontaneous tension generation was recorded isometrically via a force displacement transducer (Grass FT03) in preparations not used to study electrical activity, according to a method described previously for rat uterine strips (Parkington & Lipton, 1976).

Histological analysis was carried out on preparations having cross-sectional areas of 0·3 to 0·4 mm². They were securely pinned out to 300–400 mg tension (measured via a force displacement transducer) in a 10 ml bath of physiological solution at 37 °C, such that no shortening of the tissue could occur when the temperature or solution were changed. The physiological solution was replaced with 0·1 M-sodium phosphate buffer (pH 7·4) at room temperature. The tissue was fixed by replacing this solution with buffer containing 4 % paraformaldehyde and 1 % gluteraldehyde in the following manner. A 1 ml aliquot of fixative was added to overflow the bath every 15 s for 5 min. The solution was gently mixed between additions. This method was used to prevent excessive shrinkage in the hypertonic fixative. The tissues were fixed for 24 h before being embedded in epoxy resin. Care was taken to ensure that the blocks containing the tissues were orientated in the microtome such that the glass knife was parallel with the long axis of the muscle bundles when 0·5 μm sections were being cut. The sections were stained with Toluidine Blue at room temperature for 12 h. Two methods were used to estimate the dimensions of the smooth muscle cells.

Method 1. Two comparable areas from the middle of muscle bundles from each of ten longitudinal sections from each tissue were photographed and enlargements were prepared. The number of nuclei per print (N) and the average maximum cell diameter (D) were determined. The relative area occupied by cells (A) was obtained from the photograph by cutting and weighing. From these measurements the area of one cell in longitudinal section was estimated to be A/N μm².

If a cell is assumed to be spindle-shaped, then the area in longitudinal section is $0{\cdot}5DL$ μm², where D is maximum cell diameter and L is cell length. Since a cell is not strictly regular in its geometry it may not be equivalent to two isosceles triangles in its longitudinal section at maximum diameter. If this were the case then the factor 0·5 would be erroneous. Measurements were made to find whether an empirical factor, other than 0·5, might be applied to the product, DL, that would more accurately estimate cell area. These involved a series of determinations of diameter at regular intervals along the length of cells constructed from serial sections. From the area of the resulting series of quadrilaterals a factor of 0·558 was obtained ($n = 6$). This factor was the same for all stages of the oestrous cycle. Thus $A/N = 0{\cdot}558DL$, and $L = A/(0{\cdot}558DN)$.

Method 2. Photographs were made of longitudinal serial sections. Profiles of two individual cells from each of three preparations (proestrus, oestrus and dioestrus) were traced from all photographs in which the cells were represented, and the image of each cell in three dimensions was obtained by superimposition of the tracings. The dimensions of the reconstructed cells could be measured directly.

In estimating the resting membrane potential the results of not less than twenty acceptable impalements were combined for each tissue. The number of animals studied, and not the number of cells examined, was used in the statistical analysis of the results. The statistic quoted with each mean is the standard error based on this number; a significance level of 0·05 was used in testing (Student's t test).

RESULTS

Resting membrane potential

The resting membrane potential of tissues from animals in proestrus was $44{\cdot}8 \pm 1{\cdot}0$ mV ($n = 6$) and during oestrus it was $44{\cdot}3 \pm 1{\cdot}2$ mV ($n = 9$). The membrane potential was more negative during dioestrus ($48 \pm 9 \pm 1{\cdot}3$ mV; $n = 9$): this value is significantly different from the other two (see Table 1).

Spontaneous activity

Since the pattern of spontaneous electrical activity in the myometrium has been shown to vary with hormone pre-treatment (Marshall, 1959; Ngu & Taylor, 1973) attempts were made to correlate the patterns of spontaneous electrical and mechanical activity of the costo-uterine muscle with the stages of the oestrous cycle. An example of the spontaneous contractile activity recorded from animals during dioestrus is shown in Fig. 1*C*. There were often long periods of quiescence. Spontaneous action

potentials recorded from tissues at that stage of the oestrous cycle are shown in Fig. 1 *A* and *B*. There were often long periods in which the resting potential maintained a steady value and no action potentials were recorded. When spikes occurred, singly or in bursts, they had an overshoot of between 15 and 30 mV. The low amplitude fluctuations in the membrane potential apparent in Fig. 1 *A* were probably

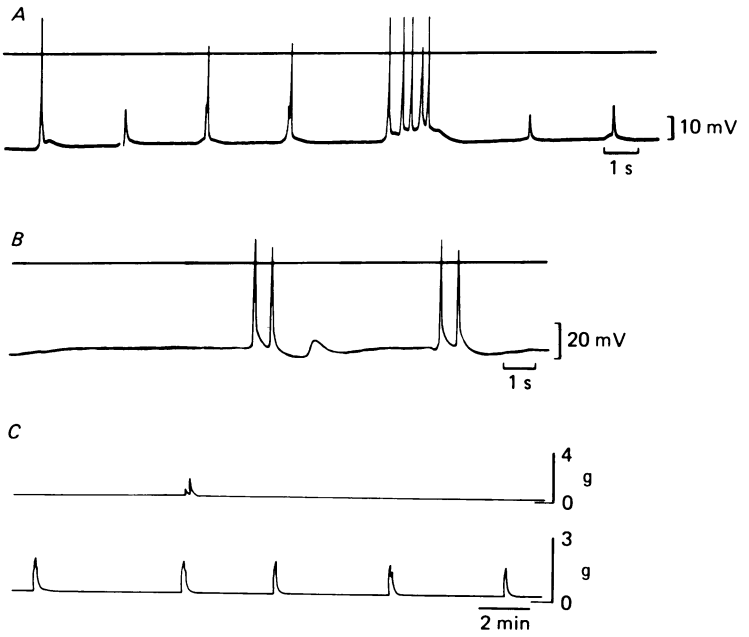

Fig. 1. The patterns of spontaneous electrical (*A* and *B*) and mechanical (*C*) activity recorded from the costo-uterine muscle of guinea-pigs in dioestrus. *A*, *B*, the top line represents zero membrane potential. The action potentials occurred singly or in bursts. Subthreshold depolarizations were also observed. *C*, contractions were of low amplitude and there were long periods of quiescence (records from two different animals).

due to the electrotonic spread of current from action potentials generated in other cells. These data correlate well with the patterns of spontaneous contractile activity that could be recorded from preparations of muscles taken from other animals in dioestrus.

Tissues from proestrous preparations were often in a state of sustained tetanic contraction as is shown in the records which were typical of this stage of the oestrous cycle (Fig. 2). Tissues from these preparations were often continuously electrically active. Action potentials occurred regularly at a frequency of about 0·3 Hz (Fig. 2 *A*). However, occasional quiescent periods of 4 to 7 s were interposed between the regular activity (Fig. 2 *B*). The action potentials at this stage of the oestrus cycle had overshoots of between 5 and 30 mV. Penetrations with micro-electrodes were well maintained. Occasionally, longer periods of quiescence of about 30 s were observed; penetrations were difficult to maintain at the beginning and end of these.

Because of the irregularity of the spontaneous mechanical activity (Fig. 3 *D*), it

was difficult to maintain penetrations in preparations from animals during oestrus. The frequency of contractions ranged from 0·1 to 1 Hz. Fig. 3*A*–3*C* shows that the action potentials occurred in bursts (with an action potential frequency of about 5 Hz) separated by periods of quiescence of 1–10 s.

Spontaneous electrical or mechanical activity was unaffected by the presence of tetrodotoxin (10^{-7} g ml^{-1}).

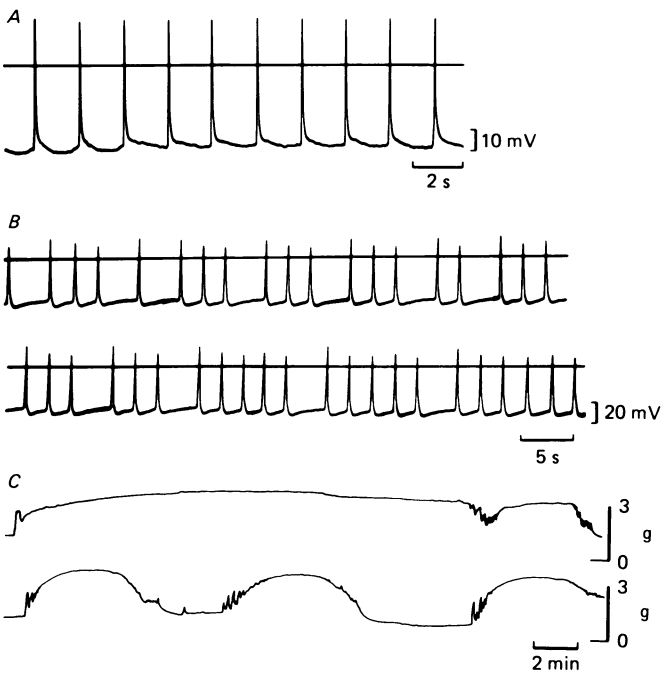

Fig. 2. The patterns of spontaneous electrical (*A* and *B*) and mechanical (*C*) activity recorded from the costo-uterine muscle of guinea-pigs in proestrus. *A*, *B*, the top line represents zero membrane potential. Action potentials usually occurred regularly at a frequency of about 0·3 Hz. *C*, tissues were often in a state of fused tetanus (records from two animals).

Passive electrical properties

Current pulses of 1·2 s duration were applied to the tissues via the large stimulating electrodes of the tissue bath and the steady-state change in the membrane potential was measured, firstly with the micro-electrode in the cell and then with it outside the cell but still within the tissue. In these experiments the artifact recorded when the micro-electrode was just outside the cell was undetectable. The field strength plotted against the steady-state change in membrane potential was found to be linear within the range +15 to −40 mV. In those tissues which were continuously spontaneously active with about 3 s between each action potential, the pulses were applied between the action potentials. Since the resting potential of the cells in most tissues seemed to be close to the threshold for the action potential, especially during

proestrus and oestrus, low field strengths were used in order to avoid inducing action potentials and movement. (A field strength between the stimulating electrodes of 50 to 150 mV cm^{-1} resulted in a steady-state deflection of the membrane potential of the order of 3–7 mV, recorded as close to the nearer stimulating electrode as possible (Fig. 4).) The mean space constant of muscles from animals in dioestrus was

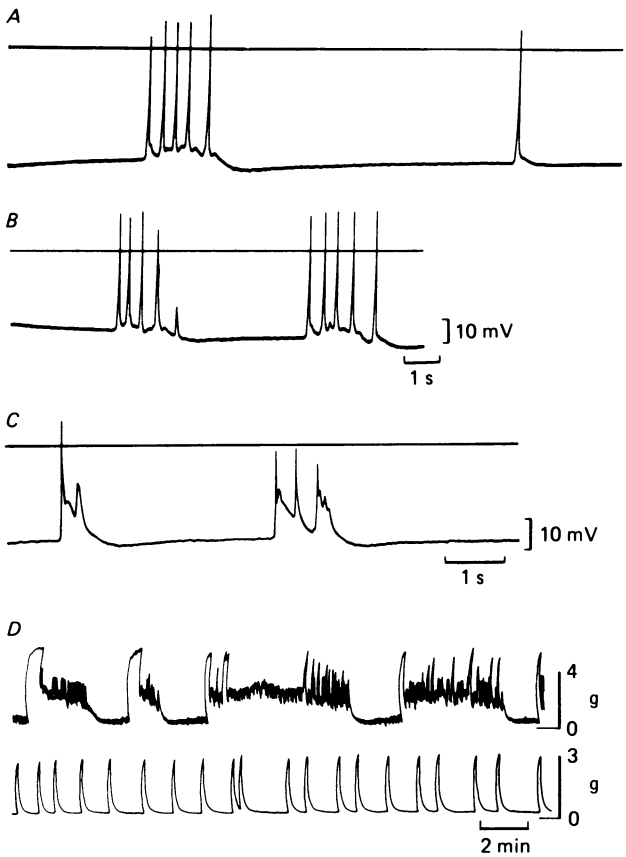

Fig. 3. The patterns of spontaneous electrical (*A*, *B* and *C*) and mechanical (*D*) activity recorded from the costo-uterine muscle of guinea-pigs in oestrus. *A*, *B* and *C*, the top line represents zero membrane potential. Action potentials occurred frequently, though irregularly. *D*, mechanical activity was frequent but irregular.

$0{\cdot}77 \pm 0{\cdot}06$ mm and this was significantly less than that obtained during proestrus ($1{\cdot}35 \pm 0{\cdot}11$ mm) or during oestrus ($1{\cdot}49 \pm 0{\cdot}11$ mm) (Table 1).

The membrane time constant was calculated according to a method suggested by Hodgkin & Rushton (1946) which has been applied to smooth muscle by many workers (see Tomita, 1970). The time for the electrotonic potential to reach half of the steady-state value was plotted against the distance from the nearer stimulating electrode. The time constant was obtained from the equation: slope $= \tau_m/2\lambda$. As shown in Table 1, the time constant during dioestrus was $209 \pm 10{\cdot}4$ ms and this was significantly lower than during either oestrus ($330 \pm 32{\cdot}3$ ms) or proestrus ($276 \pm 25{\cdot}1$ ms).

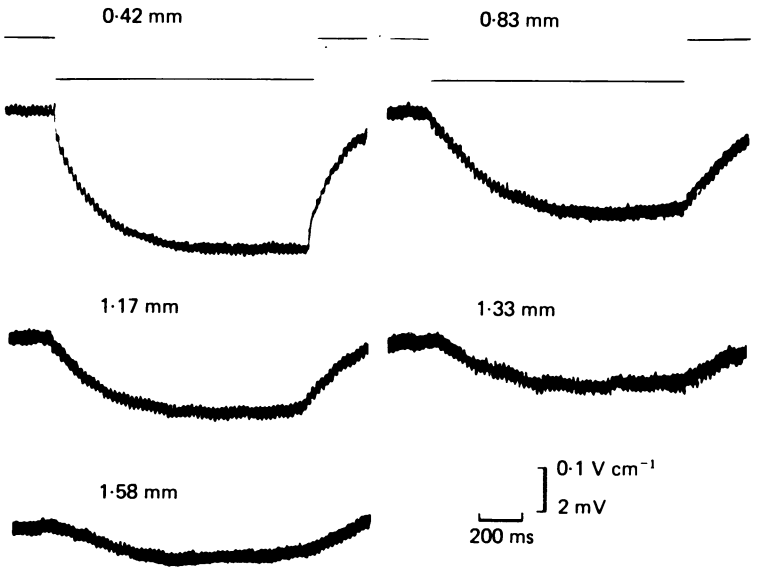

Fig. 4. The electrotonic potentials recorded in response to hyperpolarizing current pulses of 1·2 s duration at 0·42, 0·83, 1·17, 1·33 and 1·58 mm from the nearer stimulating electrode. The upper trace in each column represents the field strength between the stimulating electrodes. This was the same for all records.

TABLE 1. The passive electrical properties, resting membrane potential (r.m.p.), λ, τ_m and R_i and the smooth muscle cell dimensions of the costo-uterine muscle during proestrus, oestrus and dioestrus

	Proestrus	Oestrus	Dioestrus
R.m.p. (mV)	44·8 ± 1·0	44·3 ± 1·2	48·9 ± 1·3*
	$n = 6$	$n = 9$	$n = 9$
λ (mm)	1·35 ± 0·11	1·49 ± 0·11	0·77 ± 0·06*
	$n = 7$	$n = 11$	$n = 9$
τ_m (ms)	276 ± 25·1	330 ± 32·3	209 ± 10·4*
	$n = 7$	$n = 11$	$n = 9$
R_i (Ω cm)	112 ± 11	133 ± 20	274 ± 67*
	$n = 6$	$n = 9$	$n = 9$
D (μm)	4·77 ± 0·16	4·94 ± 0·14	3·77 ± 0·08*
	$n = 6$	$n = 9$	$n = 9$
L (μm)	280 ± 8	273 ± 5	209 ± 13*
	$n = 6$	$n = 9$	$n = 9$

Values are ± S.E. of the mean in all cases.

* Statistically significantly different during dioestrus compared with the same parameter during either proestrus or oestrus.

Changes in cell dimensions during the oestrous cycle

The space and time constants of a cable are related to membrane and axial resistance, R_m and R_i; to membrane capacitance, C_m, and to cable radius, a, as follows:

$$\lambda = \sqrt{(aR_m/2R_i)} \quad (1)$$

$$\tau_m = R_m C_m \quad (2)$$

An attempt was made to assess the possible effects of the sex steroids on the resistances R_m and R_i. It was assumed that C_m did not change throughout the oestrous cycle. This parameter has been measured in isolated smooth muscle cells from toad stomach (Singer & Walsh, 1977), oviduct (Sinback & Shain, 1979) and the giant smooth muscle cells of the invertebrate Beröe (Hernandez-Niçaise, Mackie & Meech, 1980) and was found to have a value of about 1·5 μF cm^{-2}.

The values of τ_m suggest that R_m was 185 kΩ cm^2 during proestrus, 220 kΩ cm^2 during oestrus and 139 kΩ cm^2 during dioestrus. Inserting these values of R_m and the values of λ into equation (1), for each stage of the oestrous cycle, showed that the ratio a/R_i was 13·3 (mm/Ω cm) during proestrus, 13·5 during oestrus and 5·67 during dioestrus. The difference observed during dioestrus could be due to either a decrease in the radius of the cable, a, or to a decrease in R_i.

Theoretical considerations and experimental evidence strongly suggest that the radius of the equivalent cable is the same as that of the cell in smooth muscle (Tomita, 1969). Thus an investigation of any change in the dimensions of the cells of the costo-uterine muscle throughout the oestrous cycle should reveal whether changes in R_i or in a are responsible for the changes observed in λ under the different hormonal conditions.

It has been suggested that the internal axial resistance, R_i consists of two resistances in series, the cytoplasmic resistance R_c, and the junctional resistance, R_j (Tomita, 1975): $R_i = R_c + R_j$. It has been established that the intracellular concentrations of ions do not change significantly during the oestrous cycle or in pregnancy (Casteels & Kuriyama, 1965; Bülbring, Casteels & Kuriyama, 1968). It may be assumed, therefore, that cytoplasmic resistivity, R_c, remains constant. This means that any change in R_i would have to be attributed to changes in R_j. An increase in R_j could result from a decrease in the size or the number of the low resistance pathways between the smooth muscle cells or a reduction in their conducting properties. Alternatively, such a result could also be explained in terms of a decrease in cell length such that current had to pass through a greater number of junctions in any given distance of the tissue with the high R_j, compared with a preparation in which R_j was found to be lower.

Smooth muscle cell dimensions

The smooth muscle cells of the costo-uterine muscle are approximately spindle-shaped with one centrally placed nucleus. The maximum diameter of the smooth muscle cell (found to be at the nucleus) was $3·77 \pm 0·08$ μm ($n = 9$) during dioestrus and this was significantly smaller than during oestrus ($4·94 \pm 0·14$ μm; $n = 9$) or proestrus ($4·77 \pm 0·16$ μm; $n = 6$) (Table 1). Using the equation $L = A/0·558ND$ the length of the smooth muscle cells of the costo-uterine muscle was estimated to be 280 ± 8 μm ($n = 6$) during proestrus, 273 ± 5 μm ($n = 9$) during oestrus and these values were significantly greater than during dioestrus (209 ± 13 μm; $n = 9$) (Table 1). Cell length measured in this way correlated to within 15 μm with the length of reconstructed cells (Method 2), at the same stage of the oestrous cycle (proestrus, 286 μm; oestrus, 284 μm; dioestrus, 222 μm; $n = 2$ in all cases). Although it is quite likely that shrinkage of the tissues may have occurred during the fixation procedure, this was regarded as unimportant to the result since the *comparative* values of cell

diameter and length, and not so much their absolute values, are of importance in this instance.

These calculations also provide an estimate of the extracellular space (the complement of *A*). The extracellular space within the smooth muscle bundle was calculated by weighing the cells cut out from a photograph of known area of the tissue which had been weighed. Caveoli were included as part of intracellular volume. The

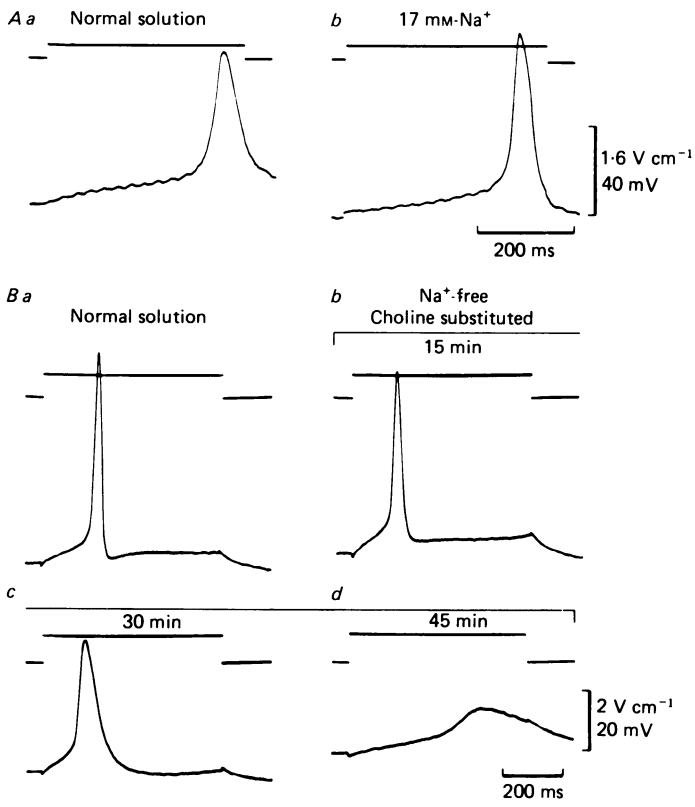

Fig. 5. The effect on the action potential of the costo-uterine muscle of replacing the sodium in the perfusing solution with choline (in the presence of 10^{-6}g ml^{-1} atropine). Upper trace: the field strength between the stimulating electrodes. Lower trace: the transmembrane response to depolarizing current pulses. *A*, the effect of replacing 130 mM-sodium with choline (30 min). *B*, the effect of replacing all of the sodium with choline.

extracellular space constituted 20–30 % (n = 18) of the smooth muscle bundles and this value did not vary significantly with changes in the oestrus cycle. Interbundle space was excluded in this calculation, since sections were examined from the middle of bundles.

The ionic basis of the action potential

It seems probable that the inward current during the upstroke of the action potential in many smooth muscles may be carried by calcium rather than by sodium (taenia coli: Holman, 1957; Abe & Tomita, 1968; vas deferens: Bennett, 1967).

However, the ionic basis of the spike in the myometrium is not clear-cut and experimental evidence suggests some sodium participation (Marshall, 1962; Kao, 1967; Osa, 1971).

Replacing 130 mM-NaCl in the solution (leaving 17 mM-$NaHCO_3$) with the isosmotic equivalent of sucrose for choline chloride resulted in an increase in the amplitude and rate of rise of the action potential (Fig. 5*A*). When all of the external sodium was replaced by either Tris, choline or sucrose, in Tris-buffered solution, for periods of in excess of 30 min all spontaneous activity ceased. (Control solution buffered with Tris had no observable effect on spontaneous activity within 30 min.) The membrane was depolarized by about 10 mV with Tris or choline substitution, although there was no change in membrane polarization when sucrose replaced sodium chloride. After about 40 min in sodium-free solution action potentials could no longer be evoked by depolarizing current pulses (an example is shown in Fig. 5*B*). When lithium was used to replace sodium the effects were similar to those observed with other substitutes but they occurred within 5 min.

Omitting calcium from the normal solution rapidly induced depolarization of about 15 mV and the amplitude of the spike was dramatically reduced. Conversely, increasing the calcium to 5 mM and 10 mM resulted in an increase in the amplitude and rate of rise of the action potential. Although most depolarizing current pulses failed to elicit a spike in the presence of verapamil (up to $2{\cdot}10^{-5}$ M), occasionally an active response was observed in respone to depolarizing current. The addition of 2 mM-manganese to the perfusing solution produced an effect similar to verapamil but 5 mM-manganese prevented any active responses to depolarizing current pulses.

DISCUSSION

The resting membrane potentials recorded from the cells of the costo-uterine muscle were comparable with that reported in the cells of other spontaneously active smooth muscles such as taenia coli (Holman, 1957; Abe & Tomita, 1968) and longitudinal myometrium (Marshall, 1962; Abe, 1971). The resting potential was highest in the longitudinal myometrium from pregnant or progesterone-treated rats (Marshall, 1962; Abe, 1971), rabbits (Kuriyama & Csapo, 1961) and guinea-pigs (Ngu & Taylor. 1973), while oestrogen treatment of these animals resulted in a reduction of the resting membrane potential. The observations from the costo-uterine muscle throughout the oestrous cycle were similar to those observed in the uterus. Flux studies suggested that progesterone treatment increases the permeability of the uterus to potassium in the rabbit (Jones, 1970), rat (Casteels & Kuriyama, 1965) and guinea-pig (Bülbring *et al.* 1968). The decrease in the frequency of the spontaneous electrical and mechanical activity during dioestrus, when there are higher levels of circulating progesterone than at any other time during the cycle, might be expected to result, at least in part, from the higher resting membrane potential observed at this time.

Action potentials of varying amplitude were recorded during dioestrus and this is perhaps best explained in terms of a depression of electrical conduction between cells. Attenuation of the spread of electrical activity might be predicted to result in contractions of lower amplitude and such a pattern has been observed in the progesterone-dominated myometrium of the mouse (Kuriyama, 1961), rabbit (Kur-

iyama & Csapo, 1961) and rat (Parkington & Lipton, 1976). Contractions of the costo-uterine muscle were of a smaller amplitude during dioestrus than at any other stage of the oestrous cycle. To estimate more quantitatively any depression of electrical conduction that might result from the presence of progesterone the space constant was estimated at the three stages of the cycle. The highest values of λ occurred in oestrus and proestrus when oestrogen levels were highest, confirming the observations made by Kuriyama & Suzuki (1976) for rat myometrium. The lowest value found for λ during dioestrus supports the hypothesis that under progesterone dominance longitudinal smooth muscle fails to contract in a co-ordinated way because of restrictions to the flow of current through the tissue. Thus any activity that might arise remains localized.

Although the effects of pre-treatment with the sex hormones on the passive electrical properties of uterine smooth muscle have been studied previously (see above), no attempt has ever been made to investigate simultaneously their effects on cell dimensions and to see whether or not the changes in electrical properties could be attributable to changes in the dimensions of the smooth muscle cells. From Table 1 it can be seen that the diameter of the smooth muscle cells of the costo-uterine muscle during dioestrus is significantly smaller than during proestrus or oestrus. This means that at least some of the decrease in λ during dioestrus can be attributed to a reduction in the diameter of the smooth muscle cells around this time. To see whether the changes in cell diameter could account for all of the differences related to the stages of the oestrous cycle values of λ, τ_m and a for each tissue were substituted into the expression for R_i; $R_i = a\tau_m/2C_m\lambda^2$ (C_m was taken to be 1·5 μF cm^{-2}, see above). The mean values for R_i are shown in Table 1. The value in dioestrus (274 Ωcm) is more than twice that of either of the others (oestrus, 133 Ωcm; proestrus, 112 Ωcm) and the difference is statistically significant in each case. Thus the differences in the ratio a/R_i are larger than can be accounted for by changes in a alone.

The increase in R_i found in the costo-uterine muscle of guinea-pigs in dioestrous is consistent with the results of Ishikawa & Bortoff (1970) who measured the total tissue resistance of rabbit longitudinal myometrium. They found that when progesterone injection followed oestrogen administration the total tissue resistance increased, compared with oestrogen treatment alone. Since $R_i = R_c + R_j$, and R_c is assumed to remain constant with changes in hormonal conditions (see above), the change in R_i in the costo-uterine muscle during the oestrous cycle is therefore attributed to changes in R_j. Because R_j estimated in the costo-uterine muscle is of the same order of magnitude as R_c estimated by Tomita (1969) it is proposed that R_j in the presence of oestrogen is neglible indicating a high efficiency of the intercellular low resistance pathways in the presence of this steroid. This might explain the ease with which activity spreads in these preparations. If it is assumed that R_c is close to the value of R_i calculated during proestrus and oestrus then R_j is about 100 Ωcm during dioestrus. This increase in R_j may be due to a decrease in the size or number of low resistance pathways between the smooth muscle cells or it could be attributed to a change in their conducting properties.

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