Electrophysiological Actions of Oxytocin on the Rabbit Myometrium

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ABSTRACT The electrical activities of myometrial cells of the pregnant rabbit uterus have been studied by means of sucrose-gap and intracellular microelectrode recording techniques. The resting potential of the myometrial cell was about −50 mv, and it is unaffected by the duration of pregnancy or placental attachment. Action potentials of the myometrium, although dependent on external Na^+, were not always of the regenerative type; preparations from nonparturient uteri often produce mainly small spikes. The mean spike amplitude was 35 mv, rising at a mean maximum rate of 3 v/sec. Oxytocin, in concentrations less than 500 μU/ml, increased the mean spike amplitude to 48 mv and the mean maximum rate of rise to 7 v/sec, without affecting the resting potential. The relation between membrane potential and dV/dt of the spike was steepened by oxytocin, suggesting that oxytocin increased the number of normally sparse sodium gates in the myometrial membrane. By this action, oxytocin is believed to increase the probability of successful regenerative spikes and thereby initiate electrical activity in quiescent preparations, increase the frequency of burst discharges, the number of spikes in each burst, and the amplitude of spikes in individual cells.

INTRODUCTION

Although it has been known for 60 years that the frequency and force of spontaneous contractions of the uterus can be increased by the octapeptide neurohypophyseal hormone, oxytocin (Dale, 1909; see Berde, 1968, for other references), it is only in recent years that attempts have been made to study the action of oxytocin on the excitatory process of the myometrium, using either external recording techniques (Jung, 1957; Marshall and Csapo, 1961), or intracellular recording techniques (Woodbury and McIntyre, 1954; Goto, 1960; Kuriyama, 1961 *a*, *b*; Kuriyama and Csapo, 1961; Marshall, 1963, 1964, 1968). The general conclusion from these studies is that in most situations oxytocin caused depolarization and thereby increased the frequency of spike discharges and hence increased contractile activities (see, for instance,

Marshall, 1964). One difficulty with most studies on the electrophysiological actions of oxytocin to date is that the doses used to produce observable effects are usually in the milliunit per milliliter range, whereas threshold contractile effects might be induced by doses in the nanounit per milliliter range (see Berde, 1968, for references).

We have reexamined the electrophysiological actions of oxytocin, using both extracellular and intracellular recordings on the pregnant rabbit myometrium (Kleinhaus and Kao, 1966, 1968; Kao, 1967; Kleinhaus, 1968). Our results, while confirming some earlier observations, differ from them in two important respects. We found no significant depolarization by microunit doses of oxytocin, and we found a new phenomenon of an increased spike amplitude in the presence of oxytocin. On the basis of external recordings made in a sucrose-gap technique, a working hypothesis was advanced that attempted to relate these two phenomena in an explanation of oxytocin action (Kao, 1967). However, since multicellular preparations were used in the sucrose-gap method, it was not possible to apply this working hypothesis with certainty to single cells. Recordings of spikes have now been made from single myometrial cells with natural resting potentials and those in which the resting potential had been altered by applied current. The time derivative of the spike (dV/dt) was also studied as an indication of the membrane current, and the findings will be interpreted in the framework of the ionic theory of excitation (Hodgkin, 1951).

It should also be pointed out at the outset that almost all the available literature on the electrophysiological actions of oxytocin cited above is based on tissues from rats, mice, and guinea pig. Two papers in the recent literature (Goto and Csapo, 1959; Kao and Nishiyama, 1964) are based on the rabbit myometrium, but neither of them dealt with the active membrane. In another paper (Kuriyama and Csapo, 1961) the active rabbit myometrium was reported to be similar to those of some other species, but no detailed evidence was given. Because of this lack of information for a species which had been used widely for contractile studies, we investigated the action of oxytocin using the pregnant rabbit myometrium. Since the action of oxytocin is primarily to modify the pattern of excitation, a brief description of the basic electrophysiological properties of the rabbit myometrium would not only facilitate an understanding of oxytocin action, but also add some new information to the understanding of the electrophysiological properties of myometrium in general. The latter objective we shall attempt to accomplish by showing in a balanced manner our observations based on extracellular and intracellular recordings. In this regard, too, our observations, while confirming some earlier findings on other species, differ from them in several other aspects.

METHODS AND MATERIALS

A. *Preparation*

The experiments were performed on isolated longitudinal muscle strips from the uterus of rabbits 25–32 days pregnant. The methods of obtaining the strips were similar to those described by Kao (1961) for the nonpregnant rabbit uterus. The preparations from the pregnant myometrium are thinner; the mean thickness estimated from the length, width, mass, and a specific gravity of 1.055 (Kao and Gluck, 1961) was 0.015 cm (Table III).

Preparations originating from interplacental regions and those overlying the areas of placental attachments were dissected and studied separately. In order to allow recovery of the membrane processes governing ionic distributions (see references in Kao, 1967), the strips were equilibrated at 25°C for about 90 min in large volumes of Krebs-HCO_3^- solution, gassed with 95% O_2–5% CO_2. Such recovered preparations were later rewarmed to 37°C for recording, except in microelectrode experiments in which spontaneous activity was not wanted.

B. *Solutions*

The Krebs-bicarbonate solution contained (in mM/liter) Na^+, 143.5; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 127.2; HCO_3^-, 25; SO_4^{2-}, 1.2; $H_2PO_4^-$, 1.2; glucose, 11.0 mM. Sodium-free solutions were prepared by replacing all NaCl with dimethyldiethanol ammonium chloride (DDA, Lorente de Nó, 1949), and all $NaHCO_3$ with choline bicarbonate (see also Kao and Gluck, 1961). In experiments in which $[K^+]_o$ and $[Ca^{2+}]_o$ were varied, additional KCl or $CaCl_2$ was added without adjusting for the tonicities. Hypertonic Krebs solution which was used to abolish spontaneous activity in the microelectrode experiments was made by incorporating 330 mM sucrose in the Krebs solution.

In experiments using a single sucrose-gap technique (Stämpfli, 1954), isotonic sucrose was prepared from glass-distilled water which was passed through a mixed bed ion exchange resin. When an estimate of the absolute membrane potential was made, one end of the muscle preparation was depolarized with an isotonic K_2SO_4 solution (121 mM/liter, Merck Index, 1960).

C. *Recordings*

The arrangement for the single sucrose-gap recording (Stämpfli, 1954) used for these experiments has been described in detail (Kao, 1967). In later sections reference will be made to "active" and "inactive" channels which denote respectively that part of the setup in which the myometrial strip was active, and that in which it was depolarized with isotonic K_2SO_4. On the active side, the incoming solutions were warmed to 37–38°C, and provision was also made for recording contractile activity with a RCA 5734 transducer tube. The active channel also contained two ring electrodes which could be used for applying electrical stimuli.

For intracellular recording, myometrial strips of about 0.5 mm width were partially pulled, through a tight fitting hole in a rubber diaphragm, into a polyethylene

tube. Built into the tube were two Ag-AgCl ring electrodes through which current pulses could be applied. Impalements were made within 2 mm of the rubber diaphragm, at which distance some extrapolar current spread could be detected. Usually, pulses were used only for stimulation. In some cases, when prepulses were used to modify membrane potential, the actual changes in the membrane potential were obtained by subtracting from the total change that portion due to extracellular IR drop caused by the interposition of the rubber diaphragm.

All recordings were made with glass capillary microelectrodes filled with 3 M KCl, having resistances of 30–60 MΩ and tip potentials of less than 5 mv. Signals were fed into a DC preamplifier with an electrometer input stage and capacity neutralizing feature (Amatniek, 1958). In most instances, for electrodes of less than 50 MΩ, the rise time can be adjusted to less than 200 μsec.

The preparations for intracellular recording were immersed in a hypertonic Krebs solution in which contractile activity was abolished even when electrical activity could be elicited by appropriate stimulation (see Tomita, 1966). The criteria used for selecting successful impalements were the same as those used by Kao and Nishiyama (1964) on nonpregnant rabbit myometrium: a rapid and clean DC shift, a stable potential for at least a few seconds, and a complete return to the base line on removal of the electrode. These criteria were independent of the observed resting potential (see Kao, 1967).

The voltage was also electrically differentiated with a RC network having a time constant of 1.1 msec. Calibration of the system was achieved by differentiating waveorms with known slopes.

RESULTS

Electrophysiological Properties of the Pregnant Rabbit Myometrium

A. RESTING POTENTIALS

1. *Values of Resting Potential* Tables I and II show the resting potential of the pregnant rabbit myometrial cell as found in the sucrose-gap method and by direct impalement. The mean resting potential is about −50 mv, and there is no statistically significant difference between the microelectrode and sucrose-gap values (Table II A). In the sucrose-gap method, there are several factors which modify the over-all potential measurement: the short-circuiting factor of the extracellular fluid, the liquid-junction potential, and some potential fall due to current flow from one end of the preparation to the other. The similarity of the sucrose-gap values to the microelectrode values suggests either that the modifying factors just balanced each other, or that the effects of these factors are relatively small as compared to the value of the membrane potential.

There are many reports in the literature to the effect that the resting potential of the myometrial cell is different at different stages of pregnancy and between the placental and interplacental portions (see, for example, Goto and Csapo, 1959; Casteels and Kuriyama, 1965; for other references, see

Kao, 1967). Our observations, summarized in Table I A and B, do not substantiate these reports. In rabbit uteri 25–32 days pregnant, we found no statistically significant difference between the resting potentials on one day as compared to another, or between placental and interplacental preparations on the same days. These observations are in good agreement with an earlier finding on nonpregnant rabbit myometrium that the mean resting potentials

TABLE I
RESTING POTENTIAL OF PREGNANT RABBIT MYOMETRIUM DETERMINED BY THE SUCROSE-GAP TECHNIQUE

A. Influence of duration of pregnancy*

Pregnancy	Resting potential
days	*mv*
25	−55.5±6.5 (3)
26	−47.3±3.5 (6)
27	−53.2±2.7 (10)
28	−51.1±1.9 (12)
29	−47.8±1.1 (13)
30	−50.4±2.7 (15)
31	−50.0±3.6 (12)

B. Comparison of placental and interplacental myometrium‡ (25–31 days pregnant)

Site	Resting potential
	mv
Placental	−51.8±3.1 (14)
Interplacental	−54.8±0.8 (24)
	$P > 0.3$

* Values in part A are based on (active side − K_2SO_4-depolarized side). All values are means ± SEM, followed by number of preparations in parentheses.

‡ Only paired pieces from the same uteri are included in part B. Some values for interplacental preparations have been used in part A.

in the estrogen-dominated and the progesterone-dominated myometrium are both about −50 mv (Kao and Nishiyama, 1964).

2. *Effects of Ions* Increasing $[K^+]_o$ consistently lowered the membrane potential of the myometrial cell. Transient bursts of spike discharge usually followed (Fig. 3 a). The relation between $[K^+]_o$ and membrane potential was linear for $[K^+]_o$ higher than 17 mM, with a slope of 32 mv for a 10-fold change of $[K^+]_o$. Although this observation is in agreement with those reported by other investigators (Goto and Csapo, 1959; Marshall, 1968; Csapo and Kuriyama, 1963; and Casteels and Kuriyama, 1965), it has no quantitative significance because $[K^+]_i$ was not constant (see discussion in Kao, 1967, p. 410).

In the sucrose-gap method, K^+-free solution did not cause any significant

change in the resting potential, although it increased spike discharges (Fig. 3 b).

Increasing or decreasing [Ca^{2+}] is reported to increase or decrease the resting potential of pregnant rat myometrium (Casteels and Kuriyama, 1965). Although in the pregnant rabbit myometrium the qualitative changes are similar, these changes occur so slowly that we cannot give precise values because of the limited long-term stability of the sucrose-gap method.

For a similar reason, the resting potential tended to become more negative in a Na^+-free solution (see, for example, Fig. 4), but the extent of hyperpolarization over a period of about 20 min was difficult to estimate.

TABLE II
RESTING POTENTIAL OF PREGNANT RABBIT MYOMETRIUM DETERMINED BY MICROELECTRODE TECHNIQUE

A. Comparison of sucrose-gap values with microelectrode values	
Sucrose-gap	−49.5±1.1 mv (92)*
Microelectrode	−48.6±1.1 mv (46)*
$P > 0.3$	

B. Effect of tonicity of medium on resting potential‡	
Solution	Resting potential
	mv
Isotonic Krebs-HCO_3	−48.6±1.1 (46)
Hypertonic Krebs-HCO_3	−47.5±0.9 (71)
$P > 0.3$	

* Mean and SEM followed by sample numbers in parentheses. Values for sucrose-gap method include all individual values used in Table I A and B.
‡ Hypertonicity produced by addition of 330 mM sucrose. Values are from microelectrode experiments representing inside-outside potentials. Numbers in parentheses indicate number of impalements in two preparations in isotonic solution, and six preparations in hypertonic solution.

3. *Effect of Tonicity* Since hypertonic Krebs solution was used in some experiments to abolish spontaneous contractions and to facilitate microelectrode impalements, it was necessary to see whether the increased tonicity had any influence on the resting potential. Table II B shows that the resting potential in a hypertonic solution is the same as that in an isotonic solution, a finding which is similar to that reported for the guinea pig taenia coli (Tomita, 1966), and that for the frog sartorius muscle (see, for example, Kao and Stanfield, 1968).

B. ACTION POTENTIAL

In the rabbit myometrium, it is virtually impossible to maintain a microelectrode properly impaled for longer than one burst of spikes. On the other

hand, extracellular recordings made with the sucrose-gap technique, although capable of showing the pattern of excitation over many bursts, cannot provide reliable information on single cell activities. Therefore, it is necessary to combine the intra- and extracellular observations for a balanced understanding of both the normal patterns of electrical activity in this tissue, and alterations therein caused by oxytocin.

1. *Normal Patterns.* (*a*) *Intracellular Recording* The unit electrical activity of the pregnant rabbit myometrium is best illustrated by spikes elicited in electrically driven preparations which had been immersed in hypertonic Krebs solution (Fig. 1). The forms of the spikes in hypertonic solution (Fig. 1 b–j) are similar to those in isotonic solution (Fig. 1 a), and spikes produced by drugs are similar to those produced by electrical stimulation.

At 37–38°C, the single spike has smooth rising and falling phases, and lasts an average of 38 msec at half-amplitude. There are wide variations in the amplitude and maximum rate of rise (Table V). Although spikes of 50–60 mv have been recorded (Fig. 1 c), many responses to electrical stimulation were not more than 10 mv (Figs. 1 d, 8 a). The repolarization phase was usually slower, but occasionally was faster (Fig. 8 c). Sometimes a transient hyperpolarization of 4–5 mv followed the repolarization of a spike (fig. 1 c, h).

In general, myometrial cells of pregnant rabbit uterus which are not actively in parturition have few spontaneous spikes, do not respond consistently to electrical stimulation, and usually produce spikes without overshoots. In parturient myometrial cells, however, there are more spontaneous spikes, and more consistent spike responses to electrical stimulation. In both, the spikes tend to be larger.

(*b*) *Extracellular Recording* The spike activities seen in a sucrose-gap recording (Kao, 1967; Kleinhaus, 1968) are usually more complex than those seen on a microelectrode recording because of the multicellular preparation used in the sucrose-gap method. The pattern of spontaneous electrical activity is best illustrated with some representative excerpts from continuous recordings made in the single sucrose-gap apparatus. Characteristically, spikes in the pregnant rabbit myometrium occur in bursts at irregular intervals (Figs. 2 a, 5, and 6). Wide variations occur in the frequency of the bursts, and the number of individual spikes in each burst (Table IV). Some preparations are quiescent, whereas others may show as many as 16 bursts per min. Similarly, whereas the spikes in most preparations tend to occur in bursts, spikes in some preparations are nearly continuous, with no distinct burst pattern.

One important feature of the electrical activity in the pregnant rabbit myometrium is that there are distinct pacemaker activities in many preparations. These can be distinguished from conducted responses (pacefollowers,

Kao, 1967) by the presence of a preceding slow depolarization similar to those seen in the heart (see Hoffman and Cranefield, 1960). Differing from the pacemakers in the heart, those in the myometrium are of two types, dis-

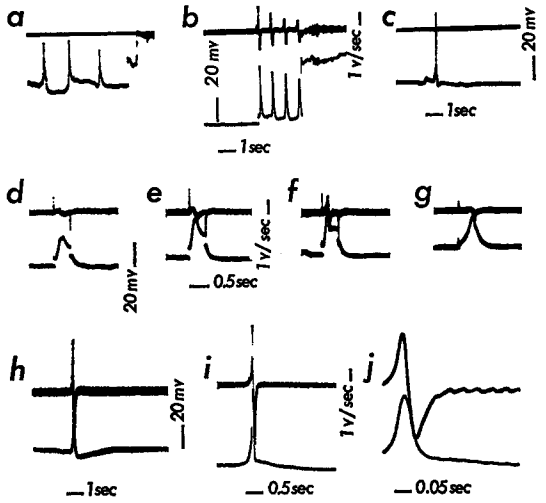

FIGURE 1. Action potentials of pregnant rabbit myometrium recorded with intracellular microelectrode. (a) was taken from myometrium in isotonic Krebs solution, all others were taken from myometrium in hypertonic Krebs solution. In all frames, bottom trace is voltage record, and top trace time derivative (*dV/dt*), except in (a) and (c) which were only reference lines. (a) 31-day pregnant myometrium. Resting potential −45 mv; microelectrode was dislodged after third spike. (b) 30-day pregnant myometrium. Resting potential −53 mv. Microelectrode dislodged after fourth spike. Note relation between spike amplitude and maximum rate of rise. (c) 29-day pregnant myometrium. Resting potential −46 mv; spike height 57 mv; note overshoot beyond reference line (top trace). (d–g) 28-day pregnant myometrium. Electrically stimulated spikes in different cells, before treatment with oxytocin (d), during treatment with oxytocin (e and f), during recovery from oxytocin treatment (g). Note the small spike before oxytocin and the slow *dV/dt* (d) and increases in both during oxytocin action (e and f). (h) 29-day pregnant myometrium. Spontaneous spike after treatment with oxytocin, 50 μU/ml. Resting potential −52 mv, spike 56 mv. Note hyperpolarization following spike. (i) 30-day pregnant myometrium. Spike elicited by electrical stimulation after treating preparation with oxytocin. Resting potential −67 mv, spike 63 mv. (j) Same preparation as (i), but different cell. Resting potential −60 mv, spike 54 mv.

tinguished from each other by their periodicity. One type has a period in tens of seconds and is responsible for burst discharges; the other with a period in tenths of seconds accounts for individual spikes in a burst (Figs. 2, 5, and 6). Quite often, apparent pacemakers fail to lead into successful spike discharges (Fig. 2 b and c), and such abortive pacemakers can be mixed in irregularly with successful pacemakers.

2. *Factors Affecting Action Potentials.* (*a*) *Stretch* When a quiescent myometrial preparation was subjected to a quick and short stretch of sufficient magnitude, it would become electrically active. The spikes thus elicited were always triggered from a prepotential of the rapid pacemaker type (Fig. 3 d and e). A quick stretch applied to an already discharging preparation will

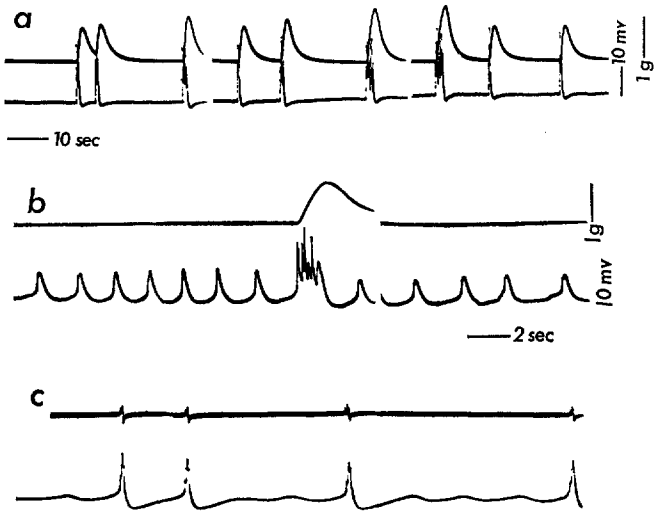

FIGURE 2. Patterns of spontaneous electrical activity of pregnant rabbit myometrium as seen in a sucrose-gap recording. In (a) and (b), top trace is contraction; bottom trace, electrical activity. (a) 29-day pregnant myometrium, placental preparation. Bursts of spikes at irregular intervals. Each burst contains many individual spikes; the number of individual spikes directly influencing the force of contraction. (b) 29-day pregnant myometrium, placental preparation. Consecutive sweeps of spontaneous activity. Note the pacemaker depolarization leading to each discharge. The first four discharges have rounded tops, the fifth, sixth, and seventh have slightly larger amplitudes and faster rates of rise. The eighth consists of complex spikes which were capable of producing contraction. The remaining discharges are similar to the first four. Except for the eighth discharge, all small discharges may represent abortive pacemakers that did not trigger regenerative spikes. (c) 29-day pregnant myometrium. Pacemaker spikes mixed with abortive pacemakers. Top trace is dV/dt.

increase the frequency of spontaneous discharges briefly. Usually, stretch affects the muscle only transiently, and as relaxation occurs the induced electrical and mechanical activities disappear. This property is used in some experiments for testing the responsiveness of the preparation.

(*b*) *Effects of* $[K^+]_o$ *and* $[Ca^{2+}]_o$ When elevated $[K^+]_o$ caused depolarization, transient bursts of action potential always occurred (Fig. 3 a).

In K^+-free medium, although no significant alteration of the resting potential occurred, spike activity usually became more frequent. This increased

activity might last for hours, as long as the preparation remained in K^+-free medium (Fig. 3 b).

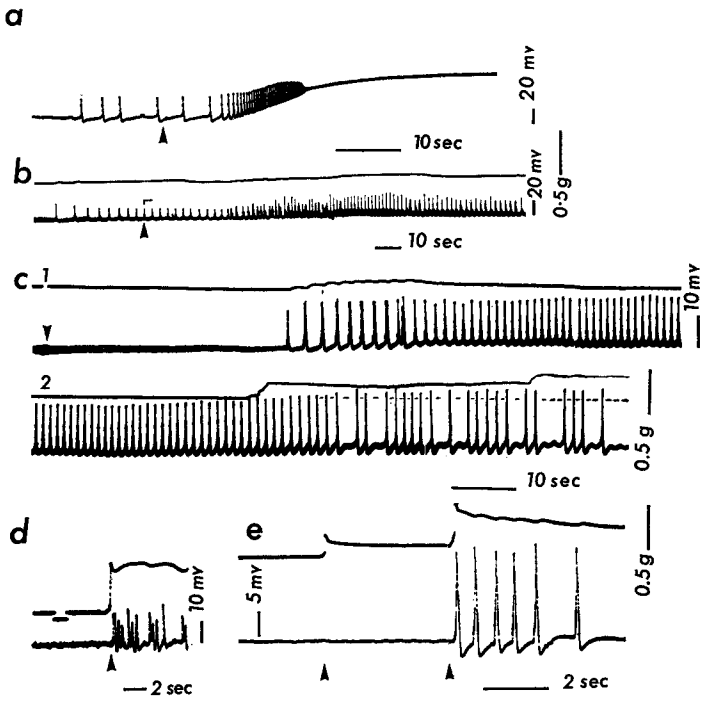

FIGURE 3. Ionic and mechanical factors affecting spike discharges in pregnant myometrium. All records were made with sucrose-gap method. (a) 30-day pregnant myometrium. Spontaneously active preparation. At arrow, 150 mM $[K^+]_o$ was introduced into the active channel resulting in depolarization and transient high frequency spike discharges. (b) 30-day pregnant myometrium, different animal. Spontaneously active preparation. At arrow, K^+-free medium was introduced. Frequency of spike discharges increased markedly, but relatively little change in resting potential was evident. Top trace is contractile record. (c) 28-day pregnant myometrium. Quiescent preparation. At arrow, Ca^{2+}-free medium was introduced. Spike discharges increased with little change in resting potential. c1 and c2 are continuous records. Top trace is contractile record. In c2, tension record was moved upward twice to avoid overlap of traces. Dotted line indicates where actual tension should be. Note that while spike frequency was still high, tension had already declined. (d) Estrogen-dominated, nonpregnant myometrium. Quiescent preparation. At arrow, quick stretch was applied, causing burst of spikes. (e) 29-day pregnant myometrium. Quiescent preparation. Two quick stretches were given (arrows), first stretch ineffective, and second stretch initiated spike discharges which in turn caused additional contractions.

When $[Ca^{2+}]_o$ was reduced to 0.2 mM, no significant depolarization was found in many preparations, nor was spike activity initiated. When a calcium-free solution was introduced, there was usually a transient period of spike discharges, followed by some depolarization (Fig. 3 c).

(*c*) *Dependence of Spikes on* $[Na^+]_o$ There is some controversy as to whether the upstroke of the action potential in the myometrium can be attributed to the inward movement of Na^+ (Daniel and Singh, 1958; Kuriyama, 1961 *a*). To study this problem, Na^+ in the Krebs solution was totally replaced by quaternary ammonium ions (dimethyldiethanol ammonium chloride and choline bicarbonate) in a series of 11 experiments on 9 preparations (Table III). As controls in Krebs solution, spike activity was initiated either by a

TABLE III
EFFECT OF SODIUM REMOVAL ON SPONTANEOUS ACTIVITY IN THE PREGNANT RABBIT MYOMETRIUM

Experiment No.*	Calculated thickness	Time for initial stimulation	Time for spike disappearance	Time for spike recovery	Maximum frequency spike/min‡	Mean frequency spike/min§
	cm	*min*	*min*	*min*		
12	—	2.28	11.92	0	85.4 (11.9)	85.4
13 a	—	1.60	4.27	—	89.5 (4.27)	89.5
13 b	—	0.23	5.0	3.75	173.6 (1.25)	70.6
15	0.007	2.88	30.60	0	117.4 (4.38)	39.0
16	0.020	3.10	4.37	—‖	93.8 (3.5)	93.8
17 a	0.007	0.80	13.73	—‖	96.8 (3.1)	49.3
17 b	—	1.03	2.98	—‖	114.7 (1.7)	71.5
17 c	0.009	0	2.53	—‖	85.3 (1.5)	60.9
19	0.025	1.62	31.2	7.5	81.7 (1.3)	42.1
20 a	0.024	0.14	10.06	0.38	127.2 (2.75)	91.2
20 b	0.015	1.66	14.3	0.46	153.6 (2.5)	102.2
Mean	0.015	1.5	11.92	2.0	111.1	72.3

* All data obtained on different strips except 13 a and b; 17 b and c.
‡ These values represent the highest spike frequency observed during wash out of $[Na^+]_o$. This frequency was maintained for the time indicated in parentheses.
§ These values indicate mean spike frequency from the time of initial stimulation to the last spike.
‖ These preparations did not recover spontaneously, but spiked immediately in response to sudden stretch.

short quick stretch or by the application of 50 μU oxytocin per ml. Such spike activity was allowed to continue for 10–20 min during which a continuous record was made. Then the Na^+-free solution was introduced without the stretch or oxytocin being applied. The sequence of events in each experiment was the same, and can be roughly divided into three phases (see Fig. 4): (i) Immediately following the change of solutions, the preparation which had been active became electrically quiescent, either immediately or after a short delay. (ii) After a variable lag, spike activity reappeared initially as closely spaced bursts, later at a relatively high frequency (Fig. 4 c–e). During this phase, the resting potential tended to become more negative, and the spike amplitude often increased. The duration of this phase is highly variable, but

towards the end, accompanying a slight decrease in the frequency (Fig. 4 f and g), the amplitude of the spikes progressively declined until all spike activity ceased (Fig. 4 h). During the initial part of this phase, the mechanical response, which had been phasic, became summated and appeared

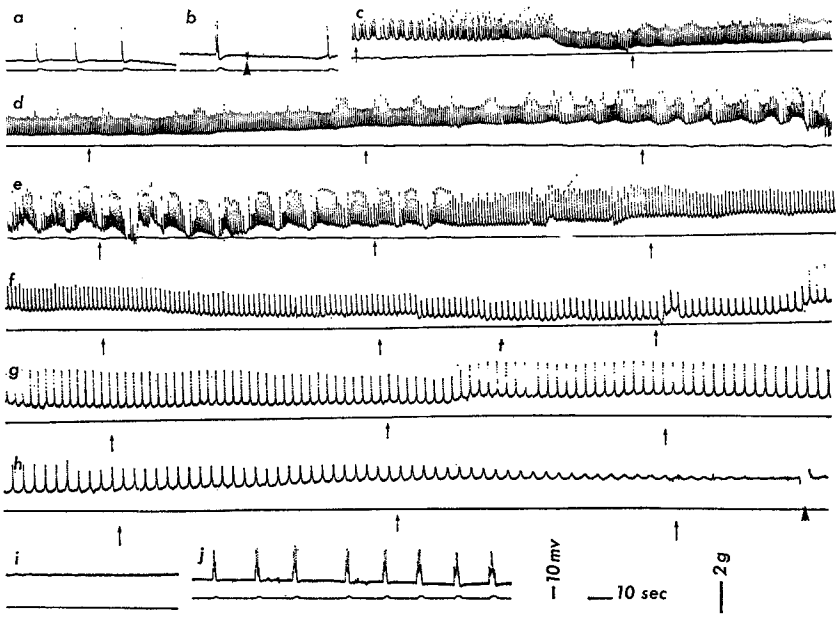

FIGURE 4. Electrical and mechanical activities of pregnant rabbit myometrium in a Na^+-free medium. Continuous sucrose-gap recording on a preparation from a 30-day pregnant uterus. Preparation was 1.8 cm long, 0.05 cm wide, and 2.5 mg in weight. (a and b) Control recording in Krebs solution. Dimethyldiethanol ammonium chloride (DDA) was introduced at large arrow in (b). (c) First small arrow marks 7.25 min after introduction of DDA, each subsequent small arrow indicates a 2 min interval. Mean spike frequency is 80/min. Tension (bottom trace) is summated. (d–h) Progressive changes in spike frequency and amplitude. In (d), mean spike frequency is 72/min. In (f) at time marked by letter *t*, tension started to fall. In (g), some hyperpolarization probably occurred. In (h), spikes stopped. At large arrow at end of (h), Krebs solution was reintroduced. (i) Quiescent latent period lasting 1.5 min. (j) Resumption of previous electrical and mechanical activities.

tetanic. However, the tension declined if high frequency spike activity remained for longer than about 10 min. (iii) Following the last remnant of spike activity, all the preparations became electrically quiescent and the tension fell further (Fig. 4 h and i). During this phase, short, quick stretches and/or oxytocin were ineffective in eliciting spike activity.

Upon reintroduction of normal Krebs solution, following a variable time lag, spikes reappeared at a frequency similar to that in Krebs solution before

the application of Na^+-free solution (Fig. 4 j). Table III summarizes all the results of this type.

Before drawing any conclusions from these experiments, two points would have to be clarified: (1) Can the cessation of spike discharges be attributed to the hyperpolarization? (2) Does the dimethyldiethanol ammonium compound have any direct toxic effect? The first point seems unlikely because artificial means of stimulation such as oxytocin, quick stretch, and electrical stimulation were all ineffective in eliciting spikes in the test solution, whereas they were all effective in normal Krebs solution. The second point cannot be readily answered because of the irresponsiveness of the preparations in the test solution. However, the rapid reversibility upon reintroduction of Na^+, sometimes within seconds, and the absence of any marked depolarization even after many minutes in the test solution indicate that no serious damage had been caused by the dimethyldiethanol ammonium compound. Some preparations did not resume spiking activity spontaneously, but responded upon a quick stretch. From tension records, it is known that the quiescence is related to the relaxed state in these preparations. On the whole, our experience with the DDA compound on the myometrium is comparable with that of Lorente de Nó (1949) on the frog nerve.

It should be emphasized that all these experiments were done in a Na^+-free solution containing 2.5 mM $[Ca^{2+}]_o$. The eventual disappearance of spikes under such conditions indicates not only that external sodium is necessary for spike production, but also that 2.5 mM of Ca^{2+} was not capable of maintaining spike activity.

Action of Oxytocin

Because the long-term stability of the sucrose-gap technique is poor, and changes in membrane potential are difficult to separate from drifts in the system, the observations to be described in this section are all gathered from continuous recordings. On many occasions in the present experiments, we have been able to produce and remove oxytocin effects without any changes in base line for periods of 30–40 min. Therefore, we feel that particular emphasis should be placed on such records because of their stability. In microelectrode experiments, it was impossible to maintain proper impalement for more than one burst of spike discharges. Therefore, it was necessary to sample many cells before and following the application of oxytocin, with particular precautions being taken to impale cells in the same regions. The validity of the conclusions drawn from repeated impalements of different cells is substantiated by observations in one extraordinary case in which the microelectrode remained properly impaled in the same cell for 9 min, during which continuous records of oxytocin action were obtained (Fig. 8).

A. RESTING POTENTIAL

As is evident from Table V, oxytocin in doses of 50–500 μU/ml does not cause any significant change in the resting potential. This point is also illustrated in Figs. 5 and 6, which show continuous recordings. Although oxytocin is capable of initiating spike activity by causing the appearance of pacemaker activity, it does not cause any lasting depolarization, because the maximum resting potential between bursts is the same as that before application or after removal of oxytocin.

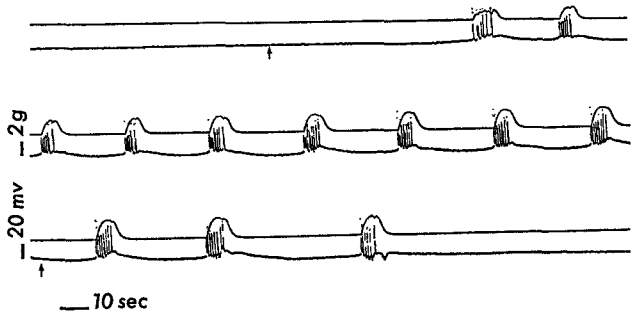

FIGURE 5. Initiation of electrical and mechanical activities by oxytocin. Continuous sucrose-gap record. 30-day pregnant myometrium, interplacental preparation. Quiescent preparation. At the arrow in the top row 500 μU oxytocin/ml was introduced. 1 min later, spikes were initiated in a characteristic burst pattern. Individual spikes increased from 6 in the first burst to 12 in the ninth burst (end of second row). Membrane potential gave characteristic swinging long-period fluctuations, but no lasting depolarization. At the arrow in the third row, oxytocin was removed. Note progressive decrease in number of individual spikes in each burst and lengthening of interval between bursts. Membrane potential after oxytocin, and at maximum negativity between bursts was the same as before oxytocin.

B. ACTION POTENTIAL

The actions of oxytocin are mainly on the action potentials and may be summarized as being fourfold: (*a*) it initiates activity in the quiescent myometrium, (*b*) it increases the frequency of burst discharges, (*c*) it increases the number of spikes in each burst, and (*d*) it can increase the amplitude of the action potentials. All these features can be seen on external recordings (Figs. 5 and 6). A proper understanding of action (*d*), however, can only be gained from recordings on single cells (Fig. 8), and an analysis of these observations will be made separately (Fig. 9).

1. *External recording.* (*a*) *Initiation of Activity* Fig. 5 shows a typical example of the ability of oxytocin to initiate electrical activity in a quiescent

preparation. Similar observations have been made on placental as well as interplacental myometrium, and on preparations taken at any time between the 25th and 31st day of pregnancy. We did not attempt to find the threshold concentration capable of initiating activity, but used 50 μU oxytocin/ml as the starting dose. Preparations which did not respond to 50 μU oxytocin/ml always responded to a higher concentration. In every instance, an effective concentration of oxytocin brought on, within several minutes, the appearance of the long-period pacemaker activity which led to burst discharges. In the continued presence of oxytocin, the interval between the bursts progressively

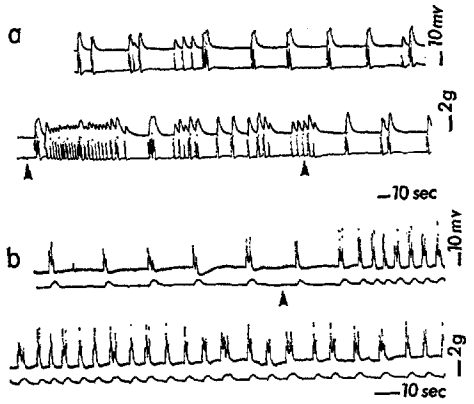

FIGURE 6. Increase of spike activity by oxytocin. Continuous sucrose-gap recordings. (a) 29-day pregnant myometrium. Spontaneously active preparation from placental region. At first arrow, oxytocin, 50 μU/ml, was introduced, leading to increased burst and spike activities. At second arrow, oxytocin was removed, followed by gradual return to original pattern of activity. (b) 29-day pregnant myometrium, placental preparation. At arrow, oxytocin, 50 μU/ml, was introduced. Oxytocin not only increased frequency of bursts and spikes, but also amplitude of the spikes. High amplitude spikes were of very short duration, suggesting the possibility of single cell origin.

shortened and the number of spikes in each burst gradually increased until a fairly stable state was reached in which bursts containing a fairly constant number of spikes appeared at regular intervals. In every preparation, the removal of oxytocin caused a lengthening of the interval between the bursts, a reduction in the number of spikes in each burst, and in many preparations a return to the original quiescent state. Such reversibility makes our observations quite different from certain claims in the literature to the effect that myometrial strips on some days of pregnancy or from the placental region are totally irresponsive to oxytocin (Csapo, 1961 *a*; Kuriyama, 1961 *a*, *b*), or that oxytocin produces a long-lasting depolarization (Jung, 1957; Kuriyama and Csapo, 1961; Marshall, 1964). Since the doses used in the other investigations were all in the milliunit ranges, the responses obtained with those high doses might represent toxic effects.

(*b*) *Increase of Burst and Spike Frequencies* Fig. 6 shows the characteristic increase of burst activity and of the number of spikes in each burst produced by oxytocin in spontaneously active preparations. A summary of these actions of oxytocin is given in Table IV.

TABLE IV
EFFECT OF OXYTOCIN ON SPIKE FREQUENCY*

A	B	C		D		E	
	Concentration of	Bursts per min		Spikes per burst		Spikes per min	
Time of pregnancy	oxytocin	Before	During	Before	During	Before	During
days	*μU/ml*						
25 (1)‡	50	15.5	21.5	4.3	3.8	64 (1)	81.5 (1)
27 (4)	50	2.8	5.3	5.6	5.6	17.9 (4)	33.7 (4)
27 (1)	100	0	9.6	0	3.7	0 (1)	34.8 (1)
28 (5)	50	0	6	0	4.8	30 (5)	50.5 (5)
28 (2)	100	4.4	11.0	3.6	4.3	45.2 (2)	51.5 (2)
29 (6)	50	2.6	5.4	12.8	20.6	38 (6)	44.7 (6)
29 (1)	100	3.1	16.0	3.0	2.4	6.2 (1)	39.0 (1)
29 (5)	500	8.5	9.9	12.2	15.1	40.6 (5)	96.2 (5)
30 (10)	50	4.7	11.6	2.7	5.2	12.7 (10)	42.8 (10)
30 (4)	100	0	3.6	0	11.3	0 (4)	41.7 (4)
30 (2)	500	0	2.5	0	10.2	0 (2)	26 (2)
Postpartum (3)	50	2.3	3.3	4.1	5	9.5 (3)	16.1 (3)
Mean (24)§	50	4.6	8.8	4.9	7.5	28.7	45.6
(7)§	100	1.9	10	1.4	5.4	12.8	41.7
(7)‖	500	4.7	6.2	6.1	12.7	20.3	61.1

* The values in the table were obtained only from experiments for which continuous records were available. Mean values for frequency in column E are frequencies for all preparations, including some preparations which did not spike in bursts. This factor accounts for some apparent discrepancies between values in column E and the product of values in columns C and D.
‡ Number in parentheses indicates number of experiments and number of preparations.
§ Mean value for frequency before the application of oxytocin includes preparations that were quiescent at the start of the experiment.
‖ Of the seven preparations, two were quiescent until treated with 500 μU oxytocin/ml, five were already spiking after prior treatment with 50 μU/ml.

(*c*) *Interaction with Ions. Calcium* There is a suggestion that the action of oxytocin is voltage-dependent, depolarizing when the membrane potential of the myometrium is more negative than the spike threshold, and repolarizing if the membrane potential is less negative than the spike threshold (Csapo, 1961 *b*; Marshall, 1964). The evidence on which this conclusion was based rested in part on lowering the membrane potential by lowering $[Ca^{2+}]_o$ (Marshall and Csapo, 1961; Kuriyama and Csapo, 1961). We repeated those experiments in nine trials on eight preparations, and failed to obtain the same results. Our findings are that when myometrial preparations were bathed in Ca^{2+}-free medium, there was a brief period of spike activity, accompanied

or followed, but not preceded, by a depolarization of 15–20 mv (Fig. 3 c). After about 20 min, these preparations became quiescent. Oxytocin, up to 500 μU/ml, did not elicit spikes in any of these preparations. Addition of 0.2

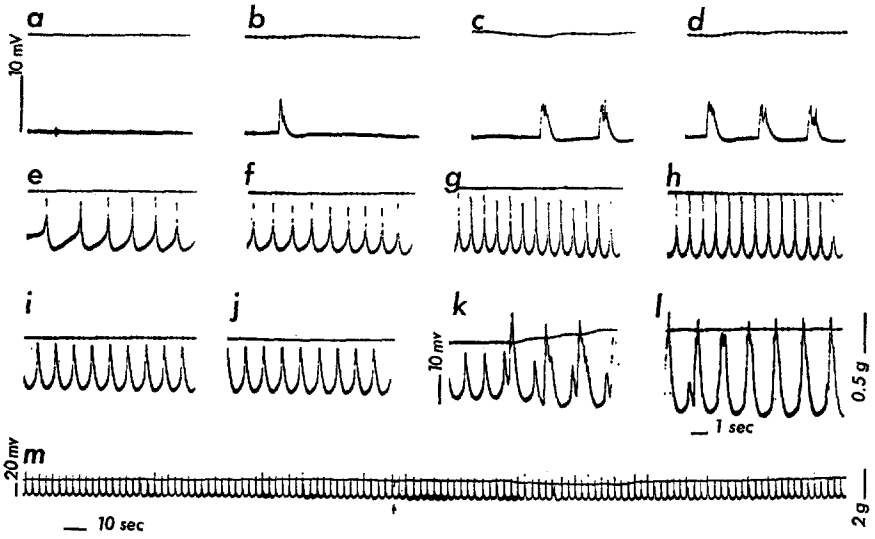

FIGURE 7. Interaction of $[K^+]_o$ and oxytocin on pregnant myometrium. Sucrose-gap recordings. (a–l) High $[K^+]_o$, (m) K^+-free. (a–l) 29-day pregnant myometrium. (a) and (b) Spontaneous activity in Krebs solution, mean spike frequency 6.2/min. Note spikes are complex because of temporal dispersion of asynchronous spikes. (c) and (d) Oxytocin, 100 μU/min. Beginning 40 sec after addition of oxytocin, (c), mean spike frequency was 39/min. After 5 min when spike frequency was stable, oxytocin was removed, and spike frequency returned to that of untreated state. (e) and (f) $[K^+]_o$ = 15 mM, frames taken 1.5 min after introduction of high $[K^+]_o$. Depolarization of 10 mv. Spikes are simple and large due to good synchronization. Mean spike frequency 45/min. (g) and (h) Oxytocin, 100 μU/ml in 15 mM $[K^+]_o$. Spike frequency 71/min. No additional change of resting potential. (i) and (j) 1 min after removal of oxytocin, $[K^+]_o$ = 15 mM. Mean spike frequency, 60/min; membrane potential unchanged. (k) and (l) 50 sec after return to 5.9 mM $[K^+]_o$. Membrane potential repolarized; synchrony of spikes beginning to be lost. Spike frequency 26/min. (m) K^+-free medium on 30-day pregnant myometrium. Spike frequency 24/min. At arrow, oxytocin, 100 μU/ml, was given. Spike frequency increased to 32/min, and membrane potential was unchanged.

mM Ca^{2+} to the medium did not cause any significant repolarization (compare Fig. 3 of Marshall and Csapo, 1961; Fig. 11 of Kuriyama and Csapo, 1961). In 0.2 mM Ca^{2+}, oxytocin did not cause any additional repolarization, nor did it initiate activity.

Potassium If oxytocin can repolarize a slightly depolarized myometrial membrane, then its effect might be manifested when the myometrium was depolarized in elevated $[K^+]_o$. Fig. 7 a–e shows one typical example of five

experiments of this type on five preparations. Details of the events can be found in the legend for that figure. All five experiments yielded similar results: the only changes in membrane potential were produced by changes in $[K^+]_o$, whereas oxytocin only caused additional changes in spike frequency without moving the membrane potential one way or the other. These observations are in agreement with Marshall's recent findings (1968) that oxytocin did not repolarize a potassium-depolarized preparation.

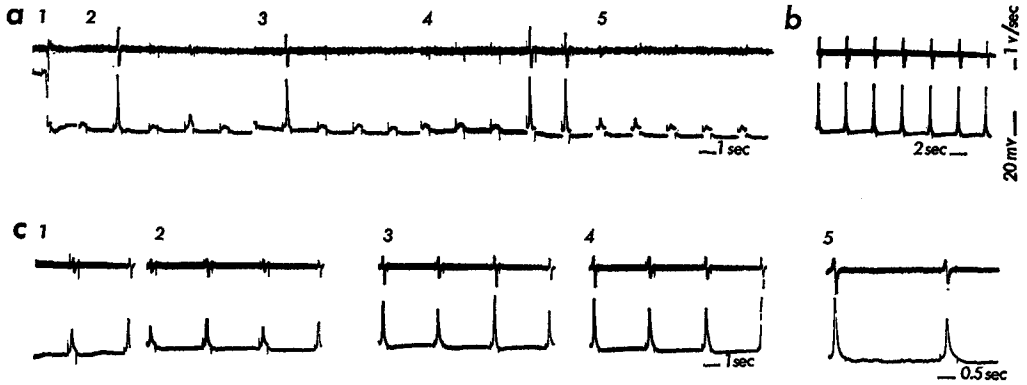

FIGURE 8. Effect of oxytocin on spike amplitude and maximum rate of rise of spike. (a) and (b) 30-day pregnant myometrium; separate impalements in the same preparation in hypertonic Krebs solution. (a) Control response in untreated state shown in five consecutive sweeps following impalement (a1) of one cell. Note that regenerative spikes were few and occurred erratically, and that some stimuli caused no response while others caused only small responses (a2 and 5). (b) 10 min after oxytocin, 25 μU/ml, was added. Note that spikes occurred in each stimulus. (c) 30-day pregnant myometrium; continuous impalement in same cell for 9 min. (c1 and 2) control responses in untreated state. (3) 2.5 min after addition of oxytocin, 50 μU/ml. (4) 3.5 min in oxytocin. (5) 7 min in oxytocin. Calibrations in right margin of b. Voltage calibration is valid for all frames. Calibration for dV/dt at 1 v/sec applies to a1–a5; for frames in b and c, same mark represents 2 v/sec.

In a K^+-free medium, myometrial preparations tend to spike frequently (24/min in Fig. 7 m). Oxytocin can cause an increased frequency (32/min in Fig. 7 m) without shifting the membrane potential.

2. *Intracellular Recording* That spike amplitude can be increased by oxytocin is evident in Fig. 6 b, a continuous sucrose-gap recording. From the rapidity of the rising phase and the short duration of the spike in other but similar cases, it has been suggested that the large spikes originated in single cells (see Kao, 1967). However, an unequivocal proof of this possibility must depend on successful intracellular recording from single myometrial cells, which will be detailed in the following.

In six preparations, the spikes initiated by electrical stimulation in the

absence and presence of oxytocin were compared. Fig. 8 shows some examples of the responses. Before the application of oxytocin, many of the cells impaled did not respond to electrical stimulation. Of the cells that did respond, many did not respond consistently, or only with rather small spikes (Fig. 8). 5–10 min after the application of oxytocin, most of the impaled cells responded to electrical stimulation, and the spike amplitude, although variable, was consistently higher than that in the untreated condition (Fig. 8 b, c). Often, spike activity occurred independently of the electrical stimulation.

Table V summarizes the resting potentials, spike amplitudes, and the maximum rates of rise in six preparations before and during treatment with oxytocin. Since the changes produced by oxytocin were variable quantitatively

TABLE V
EFFECT OF OXYTOCIN ON RESTING AND ACTION POTENTIALS OF SINGLE MYOMETRIAL CELLS OF PREGNANT RABBIT UTERI

Condition	Resting potential	Spike height	Maximum rate of rise
	mv	*mv*	*v/sec*
Untreated	−47.5±0.9 (71) [−31.6– −62.6]	34.9±2.0 (71) [6.5–64]	2.9±0.2 (64) [0.3–6.8]
Oxytocin	−47.8±0.5 (98) [−36.3– −54.6]	48.4±1.3 (106) [33.4–73.0]	7.0±0.6 (106) [2.2–34.0]
P value	>0.5	<0.001	<0.001

All values are means ± SEM followed by number of impalements in parentheses. The numbers in brackets underneath each mean value indicate the range of individual values. The same six preparations were used before and during oxytocin treatment.

from preparation to preparation, a better appreciation of the oxytocin effects can be obtained from the ranges of individual values than from the mean values. Although in the pooled data (Table V) the maximum rate of rise increased only 2.4-fold, in individual preparations it has been seen to increase nearly 10-fold. In spite of the wide variations in the individual values, the differences in the spike amplitude and maximum rate of rise with and without oxytocin were both highly significant ($P = 0.001$ in each case, Table V).

In view of this high degree of significance and the similarity in the resting potentials in the two situations, it seemed worthwhile to examine the relation between the membrane potential and the maximum rate of rise. For this purpose, all the proper impalements in which some form of response was elicited electrically, or as a result of oxytocin treatment, were taken for analysis. The natural resting potential just before a spike, or that slightly modified by a prepulse of applied current, was plotted against the corresponding dV/dt of the spike. Fig. 9 shows the results. In the absence of oxytocin,

the curve is relatively flat, with the maximum rate of rise plateauing at about 5 v/sec at resting potentials of −50–60 mv. This is a slow rate as compared with that of other tissues (see, for example, giant axon of the squid, *Loligo*, 630 v/sec (Hodgkin and Katz, 1949); giant axon of the cuttlefish, *Sepia*, 840 v/sec (Weidmann, 1951); frog sartorius muscle fiber, 450 v/sec (Nastuk and Hodgkin, 1950); skeletal muscle fibers of cat and guinea pig, 1200 v/sec (Trautwein, Zink, and Kayser, 1953); Purkinje fiber of sheep heart, 800 v/sec (Draper and Weidmann, 1951).) In the same preparations, after oxytocin

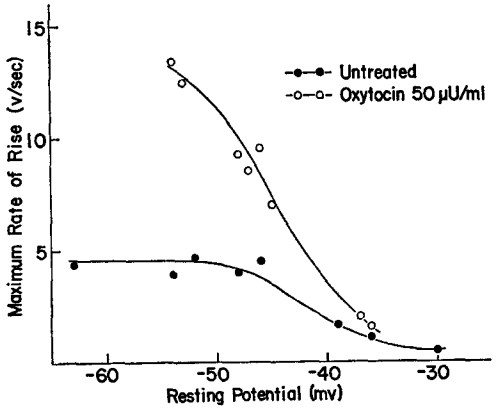

FIGURE 9. Relation between membrane potential and maximum rate of rise of spikes in untreated and oxytocin-treated preparations. Summary of data used is shown in Table V. Membrane potential is either natural resting potential or membrane potential altered by current pulse just prior to spikes for which dV/dt is plotted. Each point represents the mean of 2–14 impalements, except those for −30, −36, and −40 mv on the untreated curve and −53 mv on the oxytocin curve which are single values.

treatment the curve is steeper, a result which would adequately account for the increased spike amplitude at the same resting potential.

Other Oxytocic Agents

A few experiments with ergonovine and sparteine were performed. These compounds have actions very similar to those of oxytocin, as far as frequency of activity is concerned. We did not study the maximum rate of rise in preparations treated with these agents, but from some increased spike amplitudes we suspect that there may be some similarity to oxytocin in this respect also.

DISCUSSION

Electrophysiological Properties of the Myometrium

GESTATIONAL INFLUENCES ON RESTING POTENTIAL As cited in an earlier section (p. 761), there are claims in the literature that progesterone is responsible for a more negative resting potential in some parts of the myo-

metrium, and on different days of the pregnancy. Our failure to confirm these claims could conceivably be ascribed to the long equilibration period we used, during which the progesterone effect was lost from the isolated preparations. We find this argument unconvincing for two reasons: (*a*) we found no scientifically acceptable evidence in the literature that the resting potential of the isolated progesterone-dominated myometrium progressively declined; (*b*) the argument is contrary to the finding that another effect of progesterone, the "negative staircase" phenomenon, can persist for hours in the isolated uterus (Csapo and Corner, 1952).

SODIUM SPIKES Because of the persistence of spikes in Na^+-free media, it has been suggested that the spikes in the myometrium of some species are dependent on Ca^{2+} rather than on Na^+ (Daniel and Singh, 1958; Kuriyama, 1961 *a*). Aside from the possibility that the thickness of the preparations used prevented a proper wash out of extracellular Na^+ (Kao, 1967, p. 428), these observations are contrary to those in which both the spike amplitude and maximum rate of rise in the rat myometrium were markedly lowered in sodium-deficient media (Goto and Woodbury, 1958; Marshall, 1963). Our observations in this paper support the idea that external Na^+ is needed for spikes in the pregnant rabbit myometrium. Our evidence would be stronger if we had shown some quantitative relation between the estimated thickness and the time required for the spikes to disappear. However, the estimated thickness cannot be precise, because the small preparations often changed shape during measurement, or lost appreciable amounts of water by evaporation. We also have no information that the packing of cells and the diffusion lag are similar from preparation to preparation. Other lines of evidence in support of an essential role of Na^+ are: (*a*) in the estrogen-dominated nonpregnant rabbit myometrium, the Na^+ influx was 7–8 pM/cm²/impulse, a quantity sufficient to discharge a membrane capacity of 10 μF/cm² to 66–77 mv (Kao, Zakim, and Bronner, 1961); (*b*) in the voltage-clamped, estrogen-dominated nonpregnant rat myometrium, the early, transient inward current was reduced, and its equilibrium potential shifted towards the resting potential, when $[Na^+]_o$ was reduced (Anderson and Moore, 1968).

OXYTOCIN ACTION Our measurement of the maximum rate of rise was made with the view that in our experimental setup, at the peak of the dV/dt trace, $I_m = 0$, and that $-C_m(dV/dt) = I_i$ (Hodgkin and Katz, 1949). Since the peak of dV/dt occurs early in the myometrium, and because of recent voltage-clamp data (Anderson and Moore, 1968), it is reasonable to assume that the principal charge carrier at that time is Na^+. On this reasoning, our observations can be interpreted in the following manner: in the rabbit myometrial cell, the number of Na^+ gates capable of responding to threshold depolarization is considerably less than in many other excitable tissues. One action of oxytocin is to increase the number of such gates.

Such a mechanism could account for the various actions of oxytocin. The initiation of activity and the increase in the frequencies of burst and spike discharges could all be explained by an increased probability of successful spikes arising out of pacemaker potentials, which, in the absence of oxytocin, might have remained abortive (Fig. 2 c). The increase in spike amplitude must necessarily follow an increase in sodium gates.

Since natural oxytocin has a physiological role in parturition (for references, see Berde, 1968), it is possible that the above interpretations can also account for the rapid increase of spontaneous spike activities in the myometrium a few hours before parturition (see, for example, Kao, 1959). One point to be emphasized is that myometrial cells, unlike those of nerve axons, skeletal or cardiac muscles, are not always capable of large spikes. Under some circumstances, of which oxytocin action is one, these spikes can be induced. Therefore, to consider the over-all excitatory process of the myometrium only in terms of large spikes may be a misleading view.

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