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EXCITATION–CONTRACTION COUPLING IN VOLTAGE CLAMPED UTERINE SMOOTH MUSCLE

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SUMMARY

1. The relationship between ionic currents and contraction has been investigated in uterine strips of pregnant rat by means of a double sucrose gap apparatus combined with an optical method which permits the measurement of the contraction of the small muscular bundle where potential and current are recorded.

2. Effects of duration, size and frequency of imposed potentials upon contraction have been studied. The uterine muscle shows summation and tetanus phenomena. Tension elicited by depolarizing pulses of different durations and amplitudes can be considered as made of two components.

3. The first component of the contraction evoked by short depolarizing steps (about 50 ms) depends on the slow inward current. This contraction is abolished by manganese and lanthanum ions and by compound D 600. The amplitude of the tension can be related to the external calcium concentration and consequently to the calcium influx. The slow inward current is supposed to release a part of the bound calcium without excluding, however, a direct activation of myofibrils.

4. The second component of the contraction is observed in manganese containing solution with depolarizations longer than 200 ms and without inward current. Such a component of tension suggests the possibility of release of calcium from intracellular stores which could be located in the sarcoplasmic membrane of the uterine smooth muscle.

INTRODUCTION

The voltage clamp technique has been applied to uterine strips by Anderson (1969), by Mironneau & Lenfant (1971) and by Kao (1971). An

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inward current with slow kinetics, carried by sodium and calcium ions, is responsible for the depolarization phase of the uterine action potential. The repolarization phase is due to the decrease of the inward current and the development of an outward current (Mironneau, Lenfant & Gargouil, 1971).

A rise in intracellular calcium concentration is generally proposed as an activator of the contractile proteins in skeletal muscle (Ebashi & Endo, 1968; Sandow, 1970), cardiac (Katz, 1967) and smooth muscle (Bohr, 1964; Somlyo, Vinall & Somlyo, 1969). According to the experiments on potassium depolarized smooth muscle, it has been suggested that there are two sources of intracellular ionized calcium. One is the influx of calcium from outside in accordance with the electrochemical gradient and the membrane permeability to calcium, and the other is the release of calcium from some storage sites in the cell (Imai & Takeda, 1967). In the present experiments, the magnitude of contraction in the central compartment of a double sucrose gap preparation has been recorded by means of an optical method and the relations between contraction, membrane current and imposed potential have been investigated on pregnant rat myometrium.

METHODS

Experiments were performed on small strips of pregnant rat myometrium (after 18 days of gestation). These muscular strips were 70–120 μm in diameter and 3–4 mm in length. As previously described by Rougier, Vassort, Garnier, Gargouil & Coraboeuf (1969) for cardiac trabeculae, the preparation was placed in a double sucrose gap apparatus with an artificial node width of 50–100 μm for measurements of voltage and current. An optical method for measuring the contraction of the muscle strip in the central compartment has been used (Gargouïl, Léoty, Poindessault & Raymond, 1969). Generally, movement was confined to the part of the muscle in the central compartment and a change in the light intensity impinging on the photomultiplier was observed during the contraction.

Physiological solutions had the following compositions:

1. reference solution: NaCl 130; KCl 5·6; $CaCl_2$ 2·16; $MgCl_2$ 0·24; glucose 11 mM. The solution was aerated with O_2 and was buffered by Tris-HCl (8·3 mM) at pH 7·4.

2. the following inhibitors of permeability were used: manganese (5 mM), lanthanum (2·5 mM) and compound D 600 (α-isopropyl-α [(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl] – 3,4,5-trimethoxyphenylacetonitrile-HCl, 5×10^{-3} mM).

All solutions were maintained at $30 \pm 1°$ C.

Differences in calcium concentration were obtained by increasing (6·5 mM) or decreasing (0·72 mM) the calcium without alteration of the other constituents. EGTA (added as 1 mM solution) was used to obtain a calcium-free solution. Choline was used as a substitute for sodium after the addition of 0·1 mM atropine.

The rate of stimulation is 0·5/min.

RESULTS

Relation between contraction and action potential

Subthreshold depolarizations caused no change in the optical record. When an action potential was triggered, a modification of light intensity

occurred after a delay of 80–160 ms, and lasted 10–15 s (Fig. 1 *A*). The time course of the contraction recorded by the optical method was similar to the one observed with classical methods as proposed by Léoty & Raymond (1972). Rhythmic activity can be triggered by long lasting depolarizing

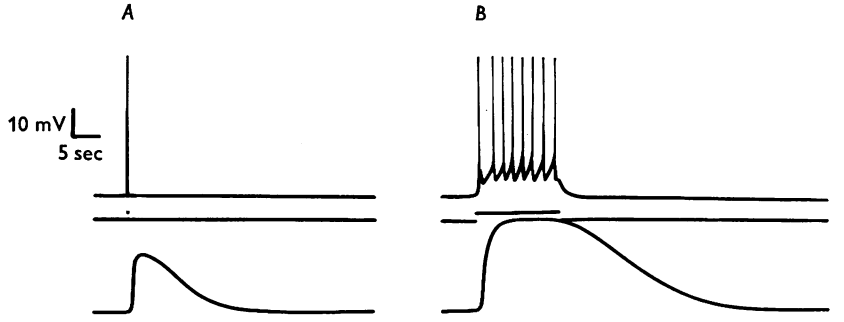

Fig. 1. Records of action potentials and contraction. *A* : contraction triggered by an action potential. *B*: a fused tetanus in response to a train of action potentials provoked by a depolarizing current pulse. The tension is expressed in arbitrary units.

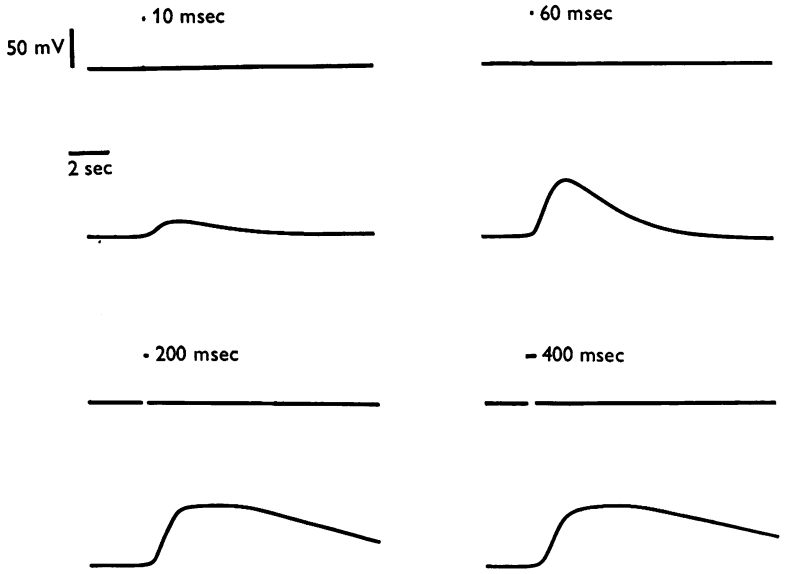

Fig. 2. Effect of different step durations upon contraction. With depolarizations longer than 200 ms, the contraction increases in duration and the declining phase becomes convex, as if there were two components of activation. However the maximum rate of rise of the contraction remains approximately constant throughout.

current steps on uterine muscle (Mironneau & Lenfant, 1972). In these conditions, the amplitude of the contraction depends on the frequency of the action potentials. If the intervals between action potentials become short enough, the tension does not revert to the resting level but summates to a fused tetanus (Fig. 1*B*). A similar tetanus which resembles that in striated muscle has been recorded previously on guinea-pig taenia coli by Bülbring (1955) and on other types of smooth muscle (West & Landa, 1956).

Effects of duration, size and frequency of potential steps upon contraction

For step depolarizations between +20 and +60 mV a transient contraction is observed even when the step duration is only 10 ms. Increasing the duration of steps leads to an increase in the magnitude of contraction (Fig. 2). The duration of the contraction, which is approximately constant for depolarizing steps shorter than 100 ms, increases for longer depolarizations. However, a second phase of increasing tension distinct from the first one can be seen.

The curve of the maximum amplitude of the contraction plotted as a function of voltage shows different shapes for different step durations between 50 and 500 ms. For a step lasting 50 ms, the contraction increases with increases in voltage from +10 mV to reach a maximum between +45 and +50 mV (Fig. 3*A*), then decreases markedly for higher depolarizations. For durations of 200 and 440 ms, the first phase of the relation between voltage and contraction is similar for voltages between +10 and +50 mV but for higher depolarizations (from +50 to +120 mV), the second phase of decrease in contraction is flatter (Fig. 3*B*, *C*). These differences between the slopes of the second part of the curves suggest that the contractile response may be considered as resulting from two components. A similar hypothesis has been put forward by West, Hadden & Farah (1951) from observations on intestinal smooth muscle in which the contraction has dynamic and tonic phases.

The effect of frequency has been recorded with trains of 50 ms depolarizations. Increasing the frequency progressively decreases the increments of the contraction while the final level of tension increases (Fig. 4). Up to 0·8 c/s contractile responses after each depolarization are still distinct and represent an incompletely fused tetanus. Over 0·8 c/s, they summate to a typical tetanus. These experiments demonstrate that uterine smooth muscle shows summation of contractions into fused tetani as a function of membrane current.

Dependence of the contraction on the slow inward current for 50 ms depolarizing steps

As stated above, depolarizations greater than +10 mV produce contractile responses the amplitude of which is dependent on the size of the imposed potential. In Fig. 5 the values of the peak amplitude of such

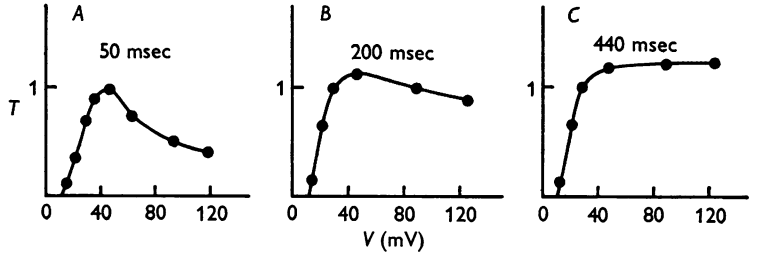

Fig. 3. Peak contraction–voltage relationship for steps of three different durations. *A*: 50 ms; *B*: 200 ms; *C*: 440 ms. The decrease of the maximum amplitude of the contraction observed for 50 ms steps higher than +50 mV becomes progressively less rapid developing into a plateau. The ordinate is expressed as a ratio of the maximum contraction obtained for 50 ms depolarizing steps.

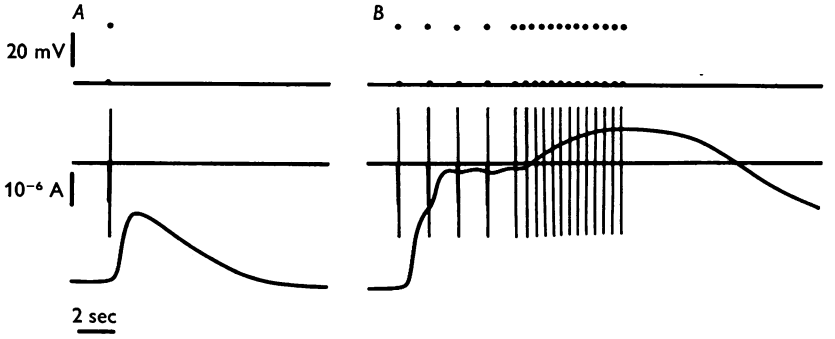

Fig. 4. Effect of inward current frequency on contraction. *A*: response to a single 50 ms depolarization. *B*: increasing the frequency decreased the increments of the contraction obtained in response to successive 50 ms steps, until a maximum level of tension was reached. Up to 0·8 c/s an incomplete tetanus can be observed. Over 0·8 c/s the contraction became a fused tetanus.

contractions and of the slow inward current (corrected for leak current by subtracting the currents obtained in manganese solution from those in the reference solution) measured at its maximum are plotted as a function of voltage. Above threshold the contraction increased in parallel with the amplitude of the inward current; after a maximum value for a depolarizing

step of about +50 mV, the tension declined but was not abolished even when the reversal potential was reached.

It has been suggested that the slow inward current of the uterine membrane is carried by both calcium and sodium ions (Anderson, Ramon & Snyder, 1971; Mironneau & Lenfant, 1971). Several substances can abolish this current. Manganese ions are known to inhibit the slow inward current of the frog heart (Rougier *et al.* 1969) and of uterine muscle (Abe, 1969; Anderson *et al.* 1971; Mironneau & Lenfant, 1971). Application of manganese (5 mM) suppressed both inward current and contraction in uterine strips (Fig. 6). Lanthanum ions are believed to block calcium regenerative systems (Hagiwara & Takahashi, 1967; Casteels, Van Breemen & Mayer, 1972) and have also been used to study the excitation–contraction coupling in rabbit aorta by Van Breemen, Farinas, Gerba & McNaughton (1972). Lanthanum ions inhibited completely the inward current and the contraction in uterine muscle for all values of depolarization. Finally, compound D 600 which is considered a highly selective substance for inhibition of calcium current (Tritthart, Grun, Byon & Fleckenstein, 1970) suppressed the contraction in uterine strips and reduced the slow inward current. A small inward current persisted, however, which may have consisted of a sodium component. In order to investigate whether the ions responsible for the slow inward current were both calcium and sodium ions, solutions with different calcium and sodium concentrations have been used. In a calcium-free solution, the contraction disappeared rapidly even with the highest depolarizations in spite of the persistence of a small inward current, thus supporting the supposition that the latter was a sodium current (Anderson *et al.* 1971) unable to induce a mechanical response. The use of solutions containing different calcium concentrations modified the contraction. A high calcium concentration (6·5 mM) led to an increase in the maximum amplitude and duration of contraction for a given depolarizing step. In a low calcium solution (0·72 mM), both amplitude and duration were reduced (Fig. 7). For these different calcium concentrations, curves depicting the peak of the contraction and the maximum amplitude of the inward current (corrected for leak current) as a function of voltage show a correlation between the mechanical response and the inward current (Fig. 8). These results seem to support the fundamental role of the calcium current in the activation of contraction and in the maximum value of tension of uterine strips. For depolarizing steps over +50 mV the decrease in the inward current was associated with a decrease in tension, even though contractions were not abolished at the reversal potential of the total inward current (calcium plus sodium), especially in the high-calcium solution. However, the presence of sodium ions as a component of the slow inward current could be relevant here.

Removal of sodium ions caused a sustained contracture in uterine strips of the kind described for the frog heart by Lüttgau & Niedergerke (1958) and for the mouse myometrium by Osa (1971). In these circumstances, in spite of the occurrence of large inward currents during depolarizing steps,

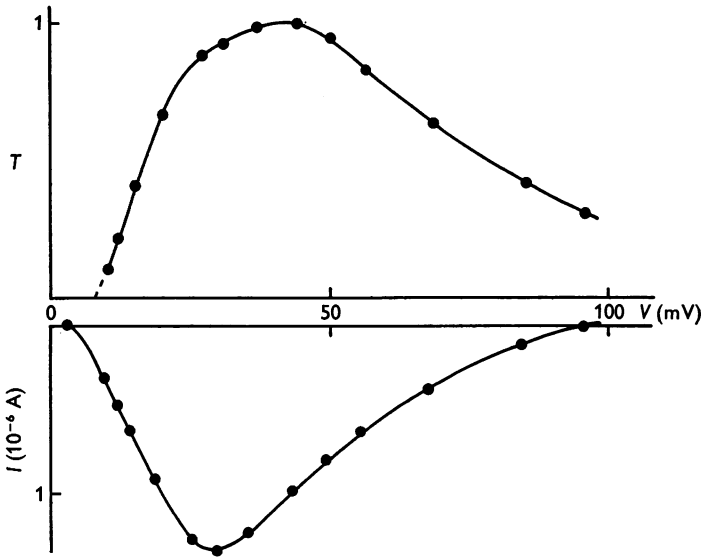

Fig. 5. Relation between the peak of contraction and of the maximum inward current as a function of voltage. The contractions were obtained in response to 50 ms depolarizing steps. The slow inward current was corrected for leak current.

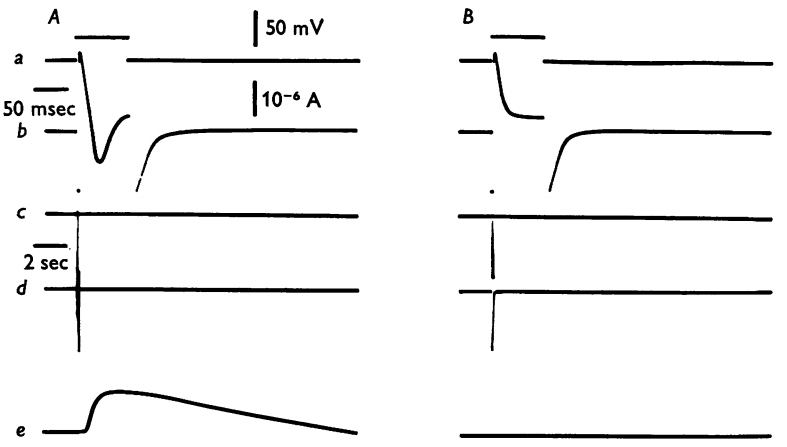

Fig. 6. Replacing a normal solution (*A*) by a manganese solution (*B*). Clamp potentials are shown at *a* and *c*, and current records at *b* and *d*. Contractions are recorded at *e*. Time base for *a* and *b*: 50 ms; for *c*, *d* and *e*: 2 s.

little or no contraction was observed. In solutions containing only 12·5 mM of sodium ions, contractions associated with depolarizations were smaller because the resting tension was already slightly elevated, but the inward currents were hardly affected. This effect of reducing extracellular sodium on contraction could be explained as due to an increase in intracellular

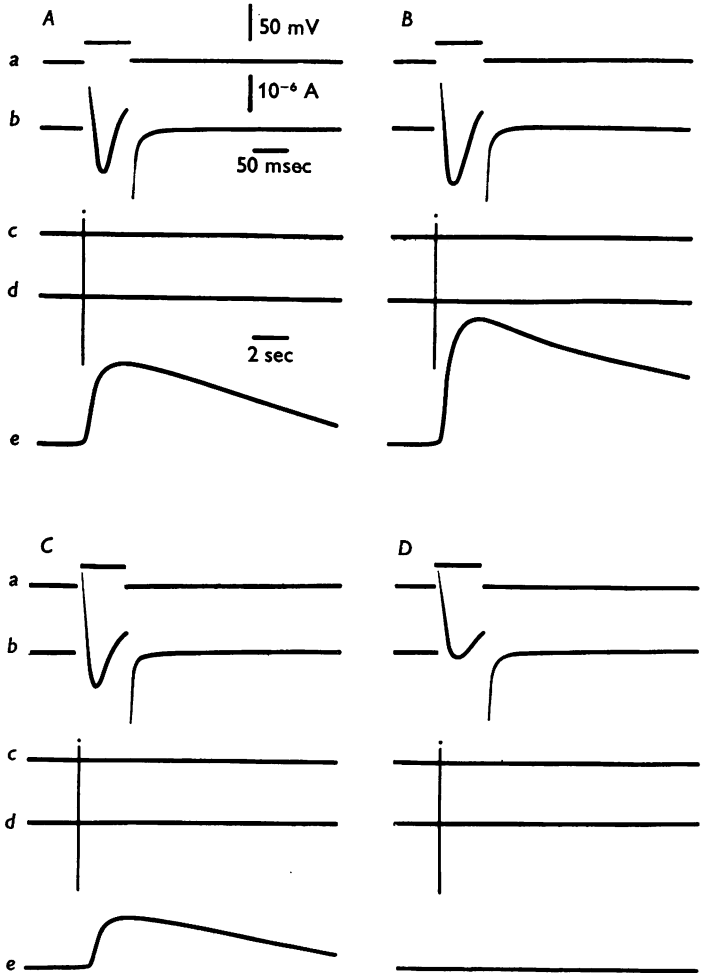

Fig. 7. Effect of different calcium concentrations on the inward current and the contraction. *A*: 2·16 mM-Ca; *B*: 6·5 mM-Ca; *C*: 0·72 mM-Ca; *D*: without Ca. A high calcium solution was associated with an increase in the amplitude and the duration of the contraction; in a low calcium solution the contraction was abolished in spite of the persistence of a small inward current. The imposed potentials and the currents were recorded as for Fig. 6. Time base: *a*, *b*: 50 ms; *c*, *d*, *e*: 2 s.

calcium concentration as a consequence of a decrease of calcium efflux, such as has been shown to occur in guinea-pig auricle (Reuter & Seitz, 1968) and in squid giant axon (Blaustein & Hodgkin, 1969).

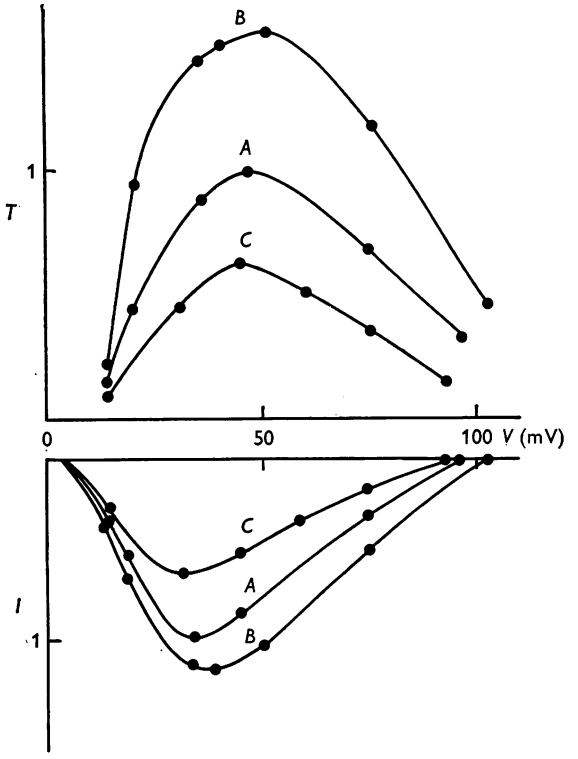

Fig. 8. Relation between the peak of contraction and the maximum inward current as a function of voltage in solutions with different calcium concentrations. *A*: 2·16 mM-Ca; *B*: 6·5 mM-Ca; *C*: 0·72 mM-Ca. The inward currents were corrected for leak current. The ordinates are expressed as a ratio of the maximum contraction and of the maximum inward current obtained in 2·16 mM-Ca.

Existence of contraction in the absence of slow inward current in manganese solution

In solutions containing manganese, normal contractile responses to brief depolarizations cannot be elicited. Nevertheless, depolarizing steps longer than 200 ms and greater than +40 mV lead to the development of tension, as shown in Fig. 9, where the onset of contraction is much delayed, often appearing only after 1 or 2 s, and its amplitude is small. Under these conditions, there is no slow inward current. The slopes of the contraction and relaxation phases are less steep than those measured in manganese-

free solution with depolarizing steps of 50 ms duration. Similar results were obtained with lanthanum ions and compound D 600. This type of contraction resembles that described on frog auricle. The relation between

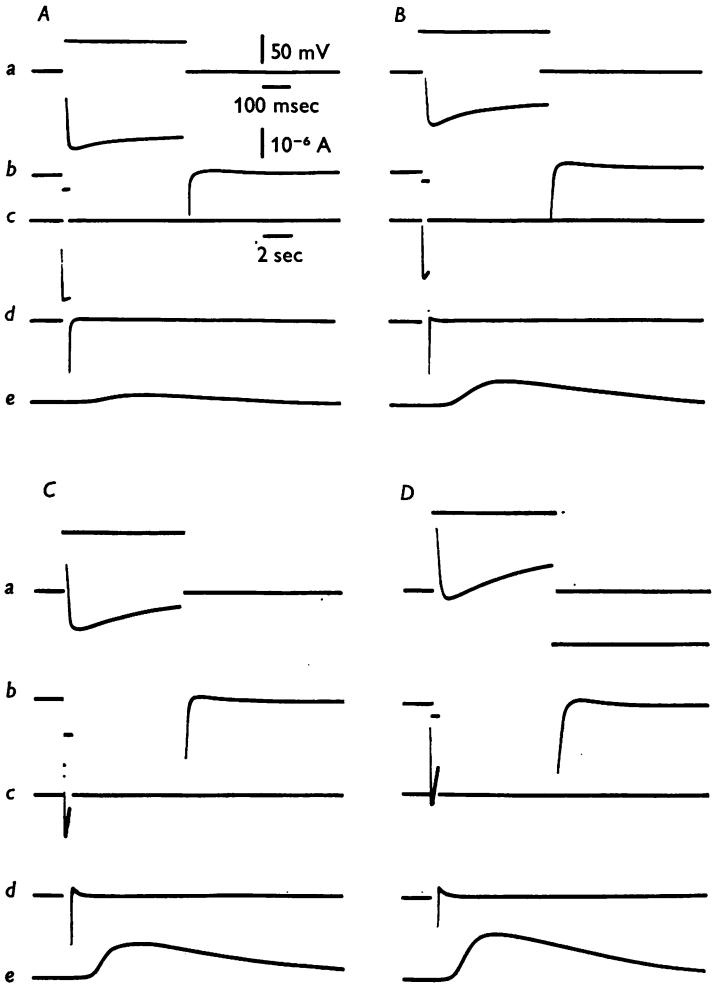

Fig. 9. Delayed contractions observed in response to long duration pulses in manganese solution. The current was always outward. The imposed potentials and the currents were recorded as in Fig. 6. Time base: *a* and *b*: 100 ms; *c*, *d*, *e*: 2 s.

peak tension and voltage has a sigmoid shape (Fig. 10*A*) as described for cardiac muscle by Morad & Orkand (1971) and by New & Trautwein (1972), and for taenia coli smooth muscle by Imai & Takeda (1967). Léoty & Raymond (1972) plotted the peak tension developed by frog auricles as

a function of the intensity of the outward current and observed a linear relationship. A similar relation between the maximum amplitude of the contraction and the outward current was obtained on rat myometrium (Fig. 10*B*).

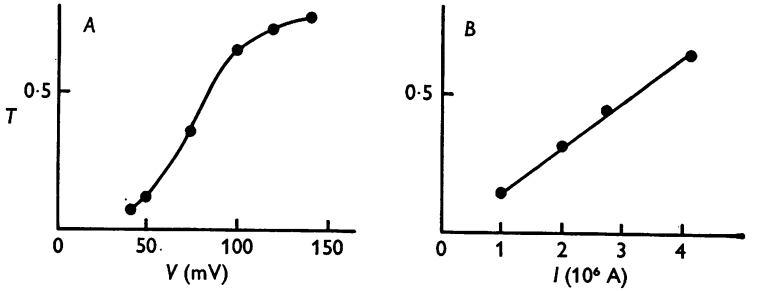

Fig. 10. *A*: peak contractions in response to 440 ms depolarizations in manganese solution plotted against voltage. The relation has a sigmoid shape. *B*: Relation between the peak of contraction and the maximum outward current. A linear relation was observed. The ordinates are expressed as a ratio of the maximum contraction obtained in response to a 50 ms depolarization in the absence of manganese.

DISCUSSION

The use of a double sucrose gap apparatus combined with an optical method permitted the simultaneous recording of both membrane currents and contractions of uterine strips taken from pregnant rats.

For depolarizing steps above threshold, contraction was recorded as a function of both inward and outward currents. Increasing the frequency of brief depolarizations demonstrated that the contractile responses of uterine smooth muscle would summate into a fused tetanus. This property has also been described for guinea-pig taenia coli (Bülbring, 1955) and rabbit and rat uterus (Csapo, 1962; Casteels & Kuriyama, 1965).

Experiments performed with different step durations and with specific inhibitors of ionic currents suggest that contractions can be activated by mechanisms of at least two distinct types: the first consisting of the slow inward current, and the second depending on some other mechanism. The identity of the first mechanism and the slow inward current is strongly suggested in the experiments for the following reasons: (1) there is a relationship between the maximum amplitude of contraction and of the inward current, both being functions of potential; (2) both contractions and inward currents in response to depolarizing steps of 50 ms duration are abolished in calcium-free, manganese and lanthanum solutions; (3) modifications of the calcium concentration are associated with alterations

of peak tension developed in response to given depolarizations. These results suggest that the contraction is related to calcium inward current and agree with those obtained on cardiac muscle (Beeler & Reuter, 1970; Ochi & Trautwein, 1971; Léoty & Raymond, 1972; Vassort & Rougier, 1972; New & Trautwein, 1972) and on smooth muscle (Kuriyama & Tomita, 1965; Tomita, 1970; Osa, 1971; Van Breemen *et al.* 1972). The minimum intracellular concentration of calcium required to evoke tension is approximately 10^{-6} M according to estimates made for frog myoplasm by Ebashi & Endo (1968). According to Kuriyama & Tomita (1965) and Casteels (1970) the calcium concentration reached in the cell approximately 10^{-5} M during depolarization could be sufficient for triggering contractions. Similar proposals have been made by Vassort & Rougier (1972) for frog auricle. On the other hand, several observations are not consistent with this hypothesis as it stands: (1) the peaks of maximum inward current and of contraction as a function of potential do not correspond; (2) there is an interval between the time at which depolarizations and contractions begin; (3) contractions occur on depolarizations to the reversal potential for the total slow inward current (calcium plus sodium). It is possible to postulate that the calcium ions which flow into the cell as a current carrier may release a portion of the calcium ions bound to intracellular sites and that it is the released calcium which activates the contractile proteins. A similar conclusion was drawn by Sakamoto & Kuriyama (1970) from experiments on muscle from the stomach of guinea-pig.

The tentative conclusion is that in response to depolarizations within the voltage range of the action potential, the contraction of uterine muscle occurs when calcium flows into the cell. Influx of calcium could either act directly on the myofibrils or indirectly by activating intracellular stores from which calcium is released. This release is promoted either by the influx of calcium or by the increase in slow inward conductance as proposed by New & Trautwein (1972). Total replacement of sodium ions by choline or sucrose produces contracture and it is generally believed that a rise in the intracellular calcium concentration is responsible. It is suggested that low extracellular sodium reduces calcium efflux. Thus calcium accumulation inside the muscle may result from impaired extrusion as well as from increased influx. Low external sodium has been associated with a reduction of calcium efflux in guinea-pig auricle (Reuter & Seitz, 1968) and in squid axon (Blaustein & Hodgkin, 1969); and with an increase in calcium content of guinea-pig taenia coli (Bauer, Goodford & Hüter, 1965).

The second component of the mechanism activating contraction, which appears with long depolarizing pulses, is different from the first since (1) it is not suppressed by manganese and (2) it is independent of inward current. It has been suggested that the diminution in amplitude and rate of rise of

tension after the suppression of the slow inward current could be explained either by preventing the filling of intracellular stores with calcium or by entering the cells (Ochi, 1970) and uncoupling the effect of calcium upon the contractile elements. Neither of these proposals, however, is consistent with the contraction which is observed in response to very long depolarizations in the presence of manganese. The existence of this second mechanism of activation (in the absence of inward current) is consistent with the hypothesis that calcium is released from intracellular stores from which it can be displaced. Since the structures of the sarcoplasmic reticulum and tubular system are poorly developed or nearly absent in the smooth muscle (Shoenberg, 1958), the intracellular stores could be located in the sarcoplasmic membrane of the uterine smooth muscle, which may have the properties of the sarcoplasmic reticulum of striated muscle, as suggested by Ito & Kuriyama (1971) and Gabella (1971).

All these results seem to support the existence of a dual source of activator calcium responsible for the activation of contractile proteins of uterine smooth muscle, as previously suggested for cardiac muscle and other smooth muscles.

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REFERENCES

ABE, Y. (1969). The effect of sodium and calcium on the action potential of pregnant rat myometrium. *J. Physiol.* **200**, 1–2*P*.

ANDERSON, N. C. (1969). Voltage-clamp studies on uterine smooth muscle. *J. gen. Physiol.* **54**, 145–165.

ANDERSON, N. C., RAMON, F. & SNYDER, A. (1971). Studies on calcium and sodium in uterine smooth muscle excitation under current-clamp and voltage-clamp conditions. *J. gen. Physiol.* **58**, 322–339.

BAUER, H., GOODFORD, P. J. & HÜTER, J. (1965). The calcium content and ^{45}calcium uptake of the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **176**, 163–179.

BEELER, G. W. & REUTER, H. (1970). The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. *J. Physiol.* **207**, 211–229.

BLAUSTEIN, M. P. & HODGKIN, A. L. (1969). The effect of cyanide on the efflux of calcium from squid axons. *J. Physiol.* **200**, 497–527.

BOHR, D. F. (1964). Electrolytes and smooth muscle contraction. *Pharmac. Rev.* **16**, 85–111.

BÜLBRING, E. (1955). Correlation between membrane potential, spike discharge and tension in smooth muscle. *J. Physiol.* **128**, 200–221.

CASTEELS, R. (1970). The relation between the membrane potential and the ions distribution in smooth muscle cells. In *Smooth Muscle*, ed. BÜLBRING, E., BRADING, A. F., JONES, A. W. & TOMITA, T., pp. 70–99. London: Edward Arnold.

CASTEELS, R. & KURIYAMA, H. (1965). Membrane potential and ionic content in pregnant and non-pregnant rat myometrium. *J. Physiol.* **177**, 263–287.

CASTEELS, R., VAN BREEMEN, C. & MAYER, C. J. (1972). A study of the calcium distribution in smooth muscle cells of the guinea-pig taenia coli using La^{3+}. *Archs int. Pharmacodyn. Thér.* **199**, 193–194.

CSAPO, A. (1962). Smooth muscle as a contractile unit. *Physiol. Rev.* **42**, 7–33.

EBASHI, S. & ENDO, M. (1968). Calcium ion and muscle contraction. *Prog. Biophys. molec. Biol.* **18**, 123–183.

GABELLA, G. (1971). Relationship between sarcoplasmic reticulum and caveolae intracellulares in intestinal smooth muscle. *J. Physiol.* **216**, 42–43*P*.

GARGOUÏL, Y. M., LÉOTY, C., POINDESSAULT, J. P. & RAYMOND, G. (1969). Evolution du courant initial global et de la contraction sur la fibre sino-auriculaire de grenouille. *C. r. hebd. Séanc. Acad. Sci., Paris* **269**, 1686–1689.

HAGIWARA, S. & TAKAHASHI, K. (1967). Surface density of calcium ions and calcium spikes in the barnacle muscle fiber membrane. *J. gen. Physiol.* **50**, 583–601.

IMAI, S. & TAKEDA, K. (1967). Actions of calcium and certain multivalent cations on potassium contracture of guinea-pig's taenia coli. *J. Physiol.* **190**, 155–169.

ITO, Y. & KURIYAMA, H. (1971). Caffeine and excitation-contraction coupling in the guinea-pig taenia coli. *J. gen. Physiol.* **57**, 448–463.

KAO, C. Y. (1971). Voltage clamp experiments on uterine smooth muscle. *Proc. XXV int. Congr. Physiol. Sci. Munich* **9**, 288.

KATZ, A. M. (1967). Regulation of cardiac muscle contractility. *J. gen. Physiol.* **50**, 185–195.

KURIYAMA, H. & TOMITA, T. (1965). The response of single smooth muscle cells of guinea-pig taenia coli to intracellular applied currents, and their effect on the spontaneous electrical activity. *J. Physiol.* **178**, 270–289.

LÉOTY, C. & RAYMOND, G. (1972). Mechanical activity and ionic currents in frog atrial trabeculae. *Pflügers Arch ges. Physiol.* **334**, 114–128.

LÜTTGAU, H. C. & NIEDERGERKE, R. (1958). The antagonism between Ca and Na ions on the frog's heart. *J. Physiol.* **143**, 486–505.

MIRONNEAU, J. & LENFANT, J. (1971). Analyse des réponses électriques de la fibre musculaire lisse d'utérus de Ratte: mise en évidence d'un courant lent calcico-sodique. *C. r. hebd. Séanc. Acad. Sci., Paris* **272**, 436–439.

MIRONNEAU, J. & LENFANT, J. (1972). Activité électrique répétitive et courant transmembranaires du faisceau musculaire lisse de l'utérus: action de l'ocytocine. *C. r. hebd. Séanc. Acad. Sci., Paris* **274**, 3269–3272.

MIRONNEAU, J., LENFANT, J. & GARGOUÏL, Y. M. (1971). L'activité électrique de la fibre musculaire lisse d'utérus: la repolarisation et les processus d'activation et d'inactivation du courant ionique sortant. *C. r. hebd. Séanc. Acad. Sci., Paris* **273**, 2290–2293.

MORAD, M. & ORKAND, R. K. (1971). Excitation-contraction coupling in frog ventricle: evidence from voltage clamp studies. *J. Physiol.* **219**, 167–189.

NEW, W. & TRAUTWEIN, W. (1972). The ionic nature of slow inward current and its relation to contraction. *Pflügers Arch. ges. Physiol.* **334**, 24–38.

OCHI, R. (1970). The slow inward current and the action of manganese ions in guinea-pig's myocardium. *Pflügers Arch. ges. Physiol.* **316**, 81–94.

OCHI, R. & TRAUTWEIN, W. (1971). The dependence of cardiac contraction on depolarization and slow inward current. *Pflügers Arch. ges. Physiol.* **323**, 187–203.

OSA, T. (1971). Effect of removing the external sodium on the electrical and mechanical activities of the pregnant mouse myometrium. *Jap. J. Physiol.* **21**, 607–625.

REUTER, H. & SEITZ, H. (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.* **195**, 451–470.

ROUGIER, O., VASSORT, G., GARNIER, D., GARGOUÏL, Y. M. & CORABOEUF, E. (1969). Existence and role of a slow inward current during the frog atrial action potential. *Pflügers Arch. ges. Physiol.* **308**, 91–110.

SAKAMOTO, Y. & KURIYAMA, H. (1970). The relationship between the electrical and mechanical activity of the guinea-pig stomach. *Jap. J. Physiol.* **20**, 640–656.

SANDOW, A. (1970). Skeletal muscle. *A. Rev. Physiol.* **32**, 87–138.

SHOENBERG, C. F. (1958). An electron microscope study of smooth muscle in pregnant uterus of the rabbit. *J. biophys. biochem. Cytol.* **4**, 609–614.

SOMLYO, A. V., VINALL, P. & SOMLYO, A. P. (1969). Excitation-contraction coupling and electrical events in two types of vascular smooth muscle. *Microvasc. Res.* **1**, 354–373.

TOMITA, T. (1970). Electrical properties of mammalian smooth muscle. In *Smooth Muscle*, ed. BÜLBRING, E., BRADING, A. F., JONES, A. W. & TOMITA, T., pp. 198–243. London: Edward Arnold.

TRITTHART, H., GRÜN, G., BYON, K. Y. & FLECKENSTEIN, A. (1970). Der Einfluss Ca^{++}-antagonistischer Inhibitoren der elektro-mechanischen Koppelung auf isolierte Uterus-Muskulatur. Untersuchungen mit der Saccharose-Trennwandteknik. *Pflügers Arch. ges. Physiol.* **319**, R 117.

VAN BREEMEN, C., FARINAS, B. R., GERBA, P. & MCNAUGHTON, E. D. (1972). Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. *Circulation Res.* **30**, 44–54.

VASSORT, G. & ROUGIER, O. (1972). Membrane potential and slow inward current dependence of frog cardiac mechanical activity. *Pflügers Arch. ges. Physiol.* **331**, 191–203.

WEST, T. C., HADDEN, G. & FARAH, A. (1951). Effect of anoxia on response of the isolated intestine to various drugs and enzyme inhibitors. *Am. J. Physiol.* **164**, 565–572.

WEST, T. C. & LANDA, J. (1956). Transmembrane potentials and contractility in the pregnant rat uterus. *Am. J. Physiol.* **187**, 333–337.