Electrical and Mechanical Interaction between the Muscle Layers of Rat Uterus and Different Sensitivities to Oxytocin

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INTRODUCTION

The muscularis externa of the uterus is composed of two muscle layers similar to that of intestine. There are two distinct considerations on the interaction between the longitudinal and circular muscle layers; one is that these layers have similar activity and other reciprocal activity. Furthermore, two different mediations between those layers are considered; a mediation by an intrinsic nervous system[1] and by myogenic conduction[2]. These suggestions were obtained mainly by the results due to the activity of intestine. In the uterine tissue, it had been reported that intrinsic nerves are rare[3]. If an interaction between those two layers in uterus is observed, the propagation of excitation may be mediate by myogenic.

In the present experiment the functional interaction of the longitudinal and circular muscle layers of the rat uterus has been examined in the longitudinal, circular and L-shaped strips. The effect of oxytocin to both muscle layers was also examined.

METHODS

The experiments were carried out on rat uterine strips of late pregnancy (12-15 days). Preparations, 2 cm in length and 3 mm in width, were prepared in the following manners: (1) longitudinal strip; longitudinal axis of the strip being parallel to that of the longitudinal direction of the horn, (2) circular strip; that of the strip being tangential to the horn and (3) L-shaped strip; details of this strip were described in the results. The preparations were mounted in an organ bath through which a solution at a temperature of 34-35°C flowed continuously. One end of the preparation was fixed and the other end was tied by a silk thread to the hook of the transducer.

Electrical activity from the longitudinal and circular strip was recorded intracellularly and extracellularly. Extracellular recordings were by pressure electrodes (3M KCl filled, 0.8–1.0 mm capillaries). Glass microelectrodes were used for intracellular recordings. A glass capillary electrode with a resistance of 50–80 MΩ, was mounted flexibly by the method of Woodbury & Brady[4]. The insertion of the electrode into a cell was controlled by a micromanipulator. The tension was recorded simultaneously with the membrane activity by means of a force-transducer. The membrane and action potentials and the deflexion of the transducer were amplified and recorded by a cathod-ray oscilloscope (DS-5016, Iwasaki) and an inkwriting oscilloscope (WI-180, Nihon Kohden). The solution was gassed with 95% O_2 and 5% CO_2. The modified Krebs solution (normal solution) contained the followings: NaCl 120.7, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.5, NaH_2PO_4 1.2 and glucose 11.5 (mM).

RESULTS

1. Electrical and mechanical activities of longitudinal muscle layer.

The segment of pregnant rat uterus was spontaneously active in normal solution. The membrane potential varied from 50 mV to 68 mV (n=32). The electrical activity appeared in a burst. One burst was consisted with many action potentials and the number of action potentials in a burst ranged from 10 to about 90. Slight depolarization during burst generation was observed in some bursts but it was not recorded in some preparations. Overshoot potential was observed especially in a few action potential of the initial phase of burst; its amplitude ranged from 5 mV to 10 mV. Fig. 1 shows various patterns of spontaneous electrical and mechanical activities recorded with intracellular microelectrodes. The duration of burst was different from preparation to preparation; it ranged from 8sec to about 50 sec. The intervals between bursts were in a similar rang during the experiment. By extracellular recordings, the burst activity of the longitudinal muscle layer could be recorded clearly. The number of action potentials consisted a burst was similar to that obtained intracellulary. Fig. 2 shows also various petterns of burst activity.

The longitudinal strip usually showed rhythmic spontaneous contractions and the burst activity was well synchronized with contraction. In typical recordings, full-size contraction with regular interval was observed (Fig. 5 & 8). However, irregular contractions were also observed in some preparations. The conduction of spontaneous excitation within the longitudinal muscle layer was measured by using two pressure electrodes.

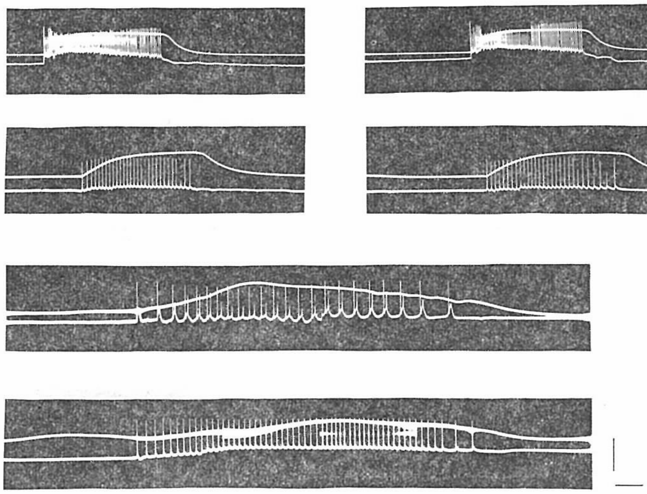

Fig. 1. Various patterns of spontaneous burst activity of the longitudinal smooth muscle.
Electrical activity was recorded intracellularly. Mechanical activity shows the phasic contraction of the longitudinal muscle layer except the last figure. Mechanical activity in the last shows the contraction of the circular muscle layer. Calibration; 10 sec and 50 mV.

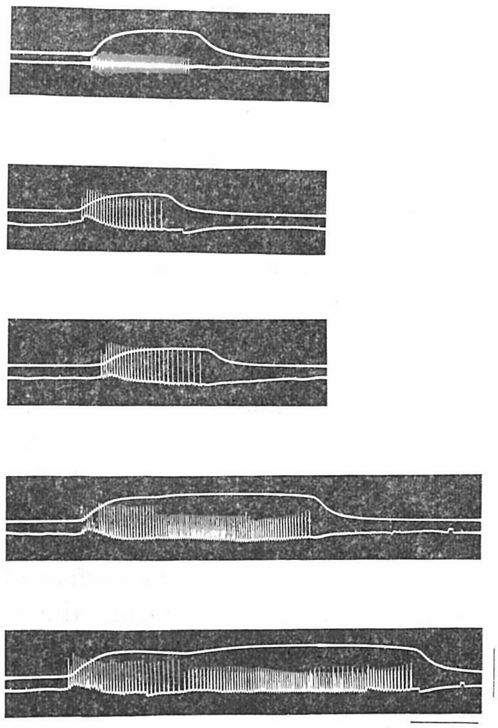

Fig. 2. Various patterns of spontaneous burst activity of the longitudianl muscle layer.
Electrical activity was recorded extracellularly. Mechanical activity shows the phasic contraction of the longitudinal muscle layer. Calibration; 10 sec and 2 mV.

These electrodes were placed at distances of 5, 8 and 20 mm apart. The excitation was conducted well thrugh the whole segment. The duration and spike number of train discharges were similar in two recordings, as shown in Fig. 3A. The conduction velocity of spontaneous excitation was estimated to be ranged from 1.3 cm/sec to 5 cm/sec. The direction of conduction of spontaneous excitation was not constant. Even in one segment, conducted from the fundus to the body of uterus in some bursts and opposite conduction was obtained in others.

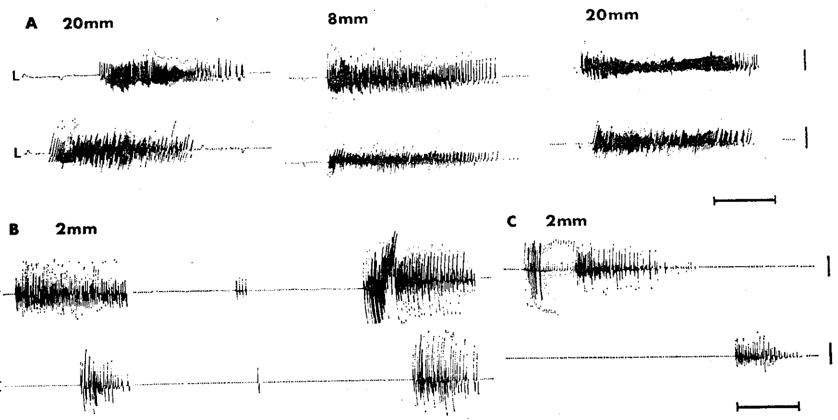

Fig. 3. Propagation of spontaneous burst activity of the muscle layers. A; Propagation in the longitudinal muscle layer in the longitudinal strip. Upper L was recorded from the nearer position to the fundus and lower L from the body. Left recording shows the propagation from the body to the fundus and right shows the converse propagation. Distances between two pressure electrodes are shown in each figure. B and C; Propagation in the circular muscle layer in the circular strip. In B, a part of train discharge was conducted. In C, no propagation of train discharge was observed. Calibration; 10 sec and 1 mV.

2. Electrical and mechanical activities of circular muscle layer.

In some experiments, a microelectrode was advanced from the endometrial side of the circular or longitudinal strip, after the mucous membrane was removed. However, in this method, fine recordings of the membrane and action potentials could not be obtained. In other experiments, the electrical activity of circular muscle layer was recorded extracellulary. The pressure electrode was placed on the surface of the endometrium of circular or longitudinal strip, without removing the mucous membrane. The electrical activity of circular muscle layer was similar to that of longitudinal one, e.g., a burst consisted with many action potentials was obtained with irregular intervals (Fig. 4).

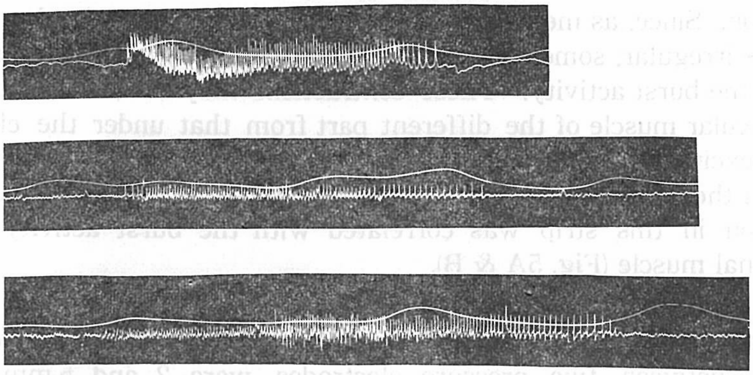

Fig. 4. Various patterns of spontaneous burst activity of the circular muscle layer. Mechanical activity shows the irregular phasic contraction of the circular muscle layer. Electrical recording was made by extracellularly. Calibration; 10 sec and 2 mV.

Contractions of the circular strip were irregular in magnitude and interval between successive contractions. As shown in Fig. 5 and 9A, the feature of contractions was very different from that of longitudinal muscle layer. Burst activity of the circular muscle was corresponded with the

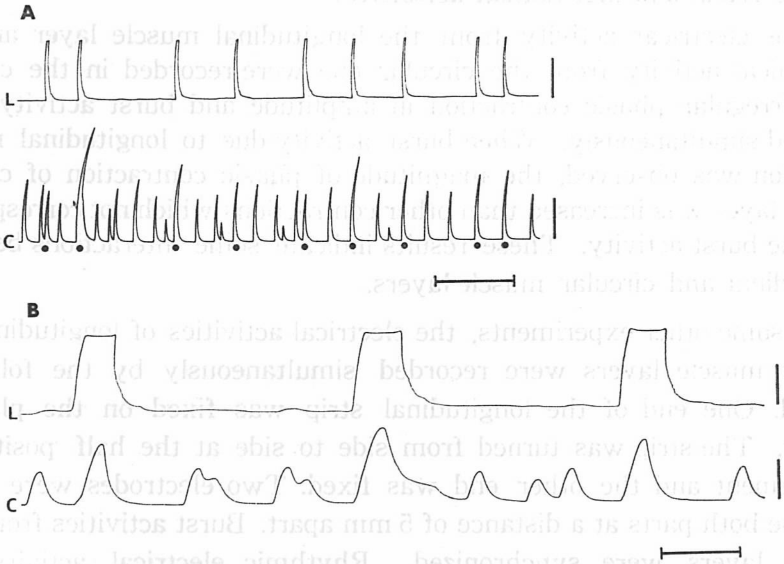

Fig. 5. Mechanical interaction between the muscle layers in the L-shaped strip. A; L and C indicate the contractions of the longitudinal and circular muscle layer respectively. Black dots in C indicate the contraction corresponded with the contraction of the longitudinal muscle. B; Similar recording as described above but recording speed is faster. Upper part of the phasic contraction of the longitudinal muscle layer was cut by saturation. Calibration; 5 min for A, 30 sec for B and 1 g.

contraction. Since, as mentioned above, the contraction of circular muscle layer was irregular, some contractions were observed without corresponding with the burst activity. These contractions may due to an excitation of the circular muscle of the different part from that under the electrode and this excitation may not propagate to the recording point. The different feature in the contraction was observed, as shown in Fig. 9E. The larger contraction in this strip was correlated with the burst activity of the longitudinal muscle (Fig. 5A & B).

The conduction of spontaneous excitation within the circular muscle layer was also measured by the same method described above. The distances between two pressure electrodes were 2 and 5 mm apart. Nevertheless these electrodes were placed closely, no conduction was observed, that is, those bursts were not synchronized. The burst durations and intervals obtained from two positions were different, as shown in Fig. 3B, C & 11A. However, in some bursts, a part of spikes in a train discharge was propagated to another recording place (Fig. 3B). As mentioned above, the conduction pattern of spontaneous excitation in longitudinal and circular muscle layer was very different.

3. Interaction between longitudinal and circular muscle layers in electrical and mechanical activities.

The electrical activity from the longitudinal muscle layer and the mechanical activity from the circular one were recorded in the circular strip. Irregular phasic contraction in amplitude and burst activity were recorded simultaneously. When burst activity due to longitudinal muscle excitation was observed, the magnitude of phasic contraction of circular muscle layer was increased than other contractions which not corresponded with the burst activity. These results indicate some interactions between longitudinal and circular muscle layers.

In some other experiments, the electrical activities of longitudinal and circular muscle layers were recorded simultaneously by the following method. One end of the longitudinal strip was fixed on the plate as usually. The strip was turned from side to side at the half position of the segment and the other end was fixed. Two electrodes were placed on those both parts at a distance of 5 mm apart. Burst activities from both muscle layers were synchronized. Rhythmic electrical activity was obtained from the longitudinal muscle layer but the electrical activity of circular one was irregular in burst duration and its interval.

An L-shaped strip was made in which each arm was composed of longitudinal and circular muscle layer with 15 mm length and 3 mm width,

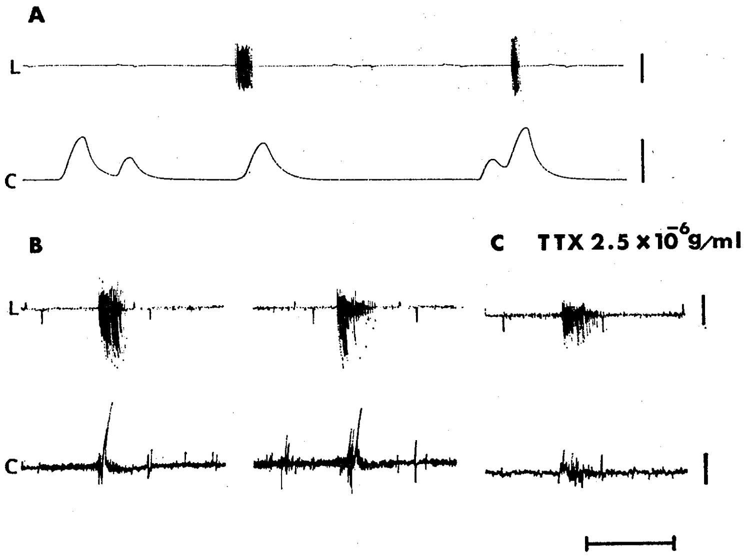

Fig. 6. Electrical and mechanical interactions between the muscle layers in the L-shaped strip.
A; L indicates the burst activity of the longitudinal muscle and C indicates the contraction of the circular muscle layer. B; L was recorded from the longitudinal muscle layer and C was obtained from the circular muscle layer. The burst interaction is observed. The distance between these two recording points was 5mm. C; After the treatment with TTX 2.5×10^{-6} g/ml, the burst interaction was still observed. Calibration; 30 sec, 1 g and 1 mV.

one end of each being left intact. The strip was mounted on the plate at the intact part. The mechanical activities were obtained from longitudinal and circular muscle layers simultaneously. As presented in Fig. 5, the intervals of spontaneous contracion in both muscle layers were different. However, some of them in circular muscle synchronized with the contraction in longitudinal muscle. Conversely, when the contraction of longitudinal muscle layer generated, larger contraction in amplitude was observed in circular muscle.

In the L-shaped preparation in which synchronization of contractions was observed, the electrical activity of longitudinal muscle layer and the mechanical activity of circular one were recorded. In this experiment, burst activity was corresponded with larger contraction in circular muscle layer (Fig. 6A). When synchronization between both mechanical activities was record, propagation of spontaneous burst activities between both muscle layers was also observed in the L-shaped preparation (Fig. 6B). The recording method in this experiment was the same as descrid in the

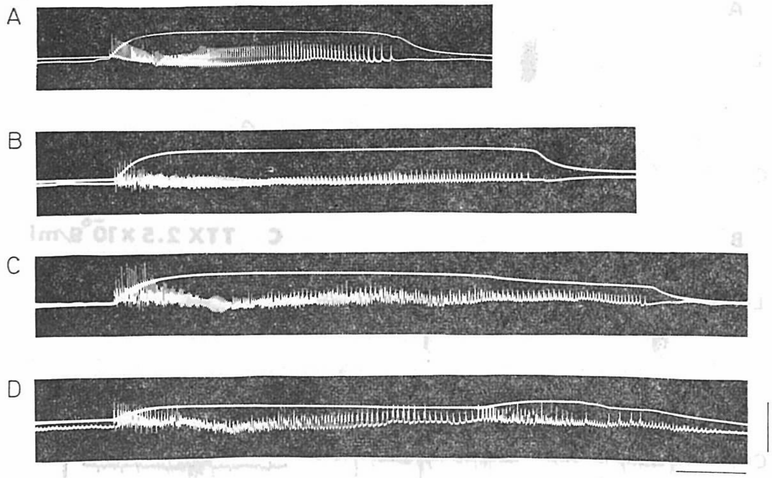

Fig. 7. Effect of oxytocin on the burst activity of the longitudinal muscle layer. A; Control, B; Oxytocin 5×10^{-5} U/ml, C; 5×10^{-4} U/ml and D; 5×10^{-3} U/ml. Electrical activity was recorded extracellularly. Mechanical activity shows the phasic contraction of the longitudinal muscle layer. Calibration; 10 sec and 2 mV.

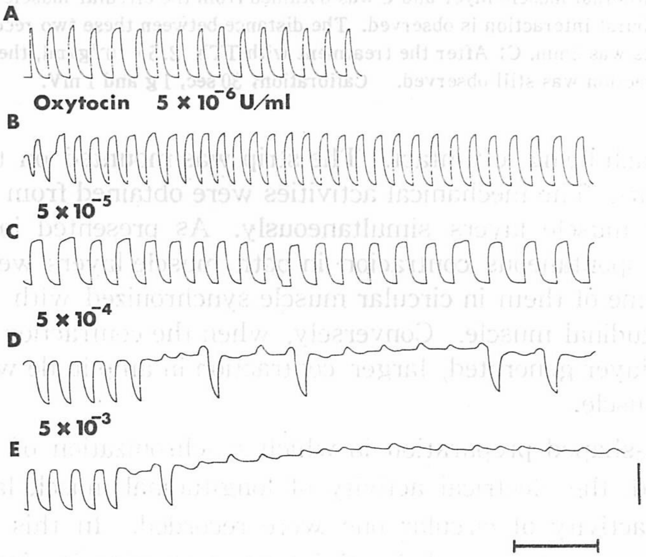

Fig. 8. Effect of oxytocin on the mechanical activity of the longitudinal muscle layer. A; Control, B; Oxytocin 5×10^{-6} U/ml, C; 5×10^{-5} U/ml, D; 5×10^{-4} U/ml and E; 5×10^{-3} U/ml. Calibration; 30 sec and 1 g.

circular strip. This synchronization was not affected by the treatment with TTX (2.5×10^{-6} g/ml) (Fig. 6C).

4. Different sensitivities of longitudinal and circular muscle layers to oxytocin.

The effect of oxytocin on the electrical and mechanical activities of longitudinal and circular muscle layers was examined. In the presence of low concentration of oxytocin (5×10^{-6} U/ml), significant changes on those activities of longitudinal and circular muscle layers were not detected. With increasing the concentration of oxytocin, the frequency and duration of burst activity of longitudinal muscle layer were increased (Fig. 7).

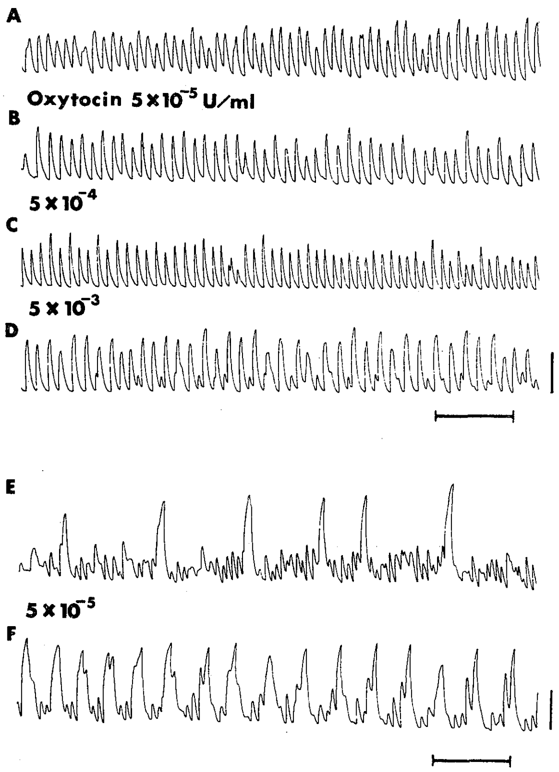

Fig. 9. Effect of oxytocin on the mechanical activity of the circular muscle layer in the circular strip.
A; Control, B; Oxytocin 5×10^{-5} U/ml, C; 5×10^{-4} U/ml and D; 5×10^{-3} U/ml. E and F were obtained by another preparation. E; Control (The feature of phasic contraction of the circular direction was different from A. Larger contraction was corresponded with the burst activity of the longitudinal muscle layer). F; Oxytocin 5×10^{-5} U/ml. Note that the frequency of larger contraction was increased. Calibration; 30 sec and 1 g.

Full-size contraction was observed in normal solution. At a concentration of 5×10^{-4} U/ml, stronger contraction which had longer duration was produced. Phasic contraction was transfered into contracture by oxytocin 5×10^{-3} U/ml (Fig. 8). Burst activity was still recorded in this solution but its duration was longer than that in normal. The resting membrane potential was not decreased and the overshoot of action potential was also observed in oxytocinl 5×10^{-8}U/ ml. In some preparations, continuous generation of action potential was recorded in the same concentration. These recordings were obrained by the longitudinal strip. On the other hand, considerable changes in the mechanical and electrical activities of circular muscle layer in the circular strip were not detected by treatment with oxytocin. Even in high concentration of oxytocin (5×10^{-3}U/ ml), burst activity and contractions were similar to those in normal solution or slightly increased (Fig. 9). The feature of contractions of both muscle layers were very different in oxytocin.

The spontaneous excitation which was observed during oxytocin 5×10^{-3} U/ml was well conducted through the longitudinal muscle layer (Fig. 10). However, the spontaneous excitation of circular muscle layer was still not conducted by treatment with oxytocin 5×10^{-3} U/ml (Fig. 11).

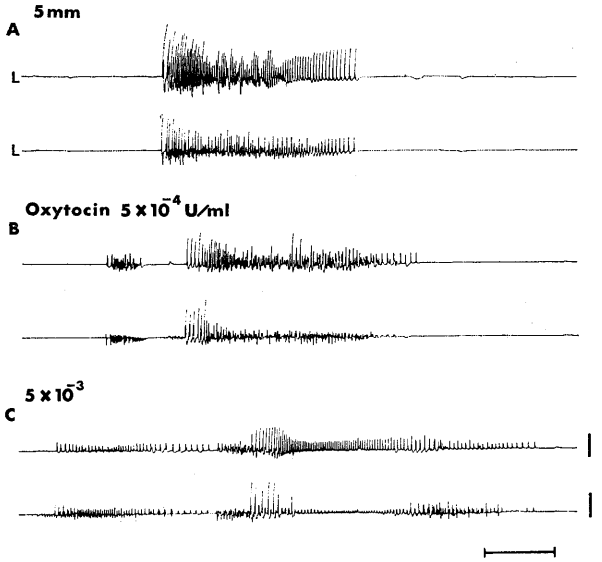

Fig. 10. Effect of oxytocin on the burst activity and the propagation in the longitudinal muscle lsyer.
A; Control. Distance between electrodes was 5mm. The duration and pattern of burst activities obtained by two electrodes were similar. B; Oxytocin 5×10^{-4} U/ml and C; 5×10^{-3} U/ml. Spikes were well conducted. Calibration; 10 sec and 1 mV.

In simultaneous recording of the electrical activities of longitudinal and circular muscle layers in the circular strip, no synchronization between those burst activities was detected in oxytocin 5×10^{-3} U/ml.

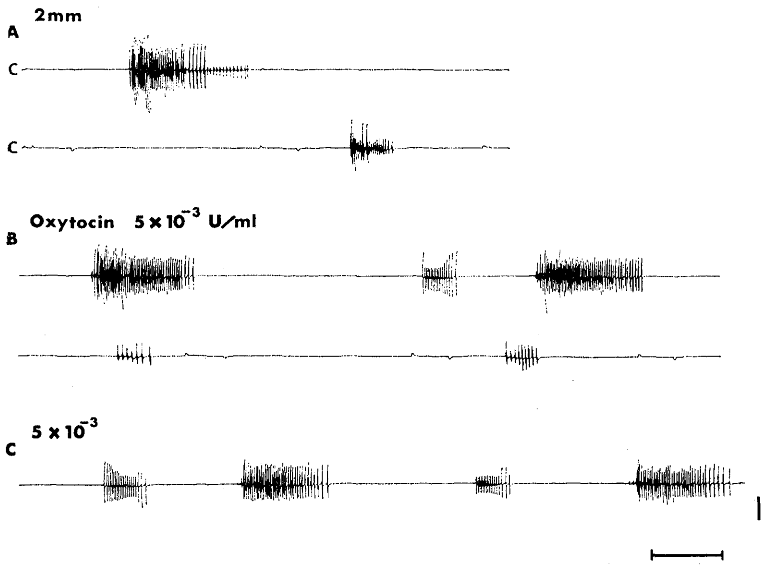

Fig. 11. Effect of oxytocin on the burst activity and the propagation in the circular muscle layer.
A; Control. Distance between electrodes was 2 mm. There are no synchronization between bursts and the duration and pattern of bursts are different.
B; Oxytocin 5×10^{-3} U/ml. No considerable change in burst activity was observed but the different pattern in burst activity was generated, as shown in B(upper C) and C. Calibration; 10 sec and 1 mV.

DISCUSSION

In the longitudinal strip, the spontaneous excitation of the longitudinal muscle layer was well propagated through the segment. Burst durations and spike numbers in train discharges were similar in the recordings obtained by two electrodes. Both directional conductions of excitation were obtained. The conduction velocity in the longitudinal axis seems to be faster than that in the circular one. On the other hand, the propagation in the circular muscle layer was very limited in the circular strip, because, in usually, no synchronization of burst activity was observed but some action potentials in a train discharge could be conduct. The features in contraction of the layers were different. A full-size contraction was usually generated in the longitudinal muscle while variable contractions in magnitude were occurred in the circular one.

These results suggest that the different function of propagation in each layer due to the differences in the characteristics of functional bundle and in the electrical resistance between adjacent smooth muscles of the muscle layers. The conduction velocity and length constant in circular muscle of pregnant mouse uterus are smaller than those in longitudinal muscle[5]. However, the conduction in the layers of small intestine was little different[2,6,7,8]. The multiple pace-maker regions in circular muscle layer were considered from the feature of contraction. Synchronization of excitation between longitudinal and circular muscle layers was obtained in the L-shaped strip. When the burst activity of longitudinal muscle layer generated, larger contraction of circular muscle layer was occurred in the circular strip. These findings were similar to those obtained by mouse uterus[5]. From these findings, it would seem likely that the layers are coupled together by some interactions. The direction of conduction was not obviously in the present experiment. However, it seems that the excitation in the longitudinal muscle layer is likely propagated into the circular layer, because a short burst activity in circular muscle was observed during a train discharge in longitudinal layer and the burst activity in the former was preceded by it in the latter. The propagation between the layers might not be mediate by an intrinsic nervous system. This suggestion is supported by the following findings; (1) the distribution of the intrinsic nerve in the muscuralis externa of uterus was very rare[3], (2) the conduction was not blocked by the treatment with TTX. Therefore, the propagation due to low electrical resistance between the layers might be considered.

By the treatment with oxytocin, the burst activity of the longitudinal muscle was increased while no considerable changes in the burst activity and contraction of the circular one were obtained. Slight increase in burst activity was observed in some circular strips.

The circular muscle seems to have less sensitivity to oxytocin. As reported previously, the circular muscle in intestine and uterus has lower sensitivity to various agents than the longitudinal muscle[1,9,10]. Brownlee et al[10] had been reported that the differences of the layers in the response to drug were in the intrinsic properties of the smooth muscle cells. When the electrical activity of the longitudinal muscle was increased by oxytocin, the potentiated activity was propagated through the longitudinal direction. However, the potentiation in propagation in the circular muscle layer was not observed in oxytocin.

In the present experiment, the preparation of longitudinal, circular and L-shaped strips were used. In the past, various methods have been employed to distinguish the motility of the layers of various organs and to find out the interaction between these muscle layers. The question is remained whether the different methods modify the nature of interaction. Further studies will be required to elucide it. It is concluded that the layers of rat uterus are coupled together by some electrical means and the properties of the longitudinal and circular muscle cells are different in electrical and in sensitivity to agents.

SUMMARY

The present experiment was carried out to investigate the interaction between the electrical and mechanical activities of longitudinal and circular muscle layers of the pregnant rat uterus. The following results were obtained.

1. The electrical activity of longitudinal and circular muscle layers was appeared in a burst which was corresponded with contraction. The features on th mechanical activity of those two layers were very different. Full-size contractions in the longitudinal layer and phasic contractions with various magnitudes in the circular one were obtained.

2. Spontaneous excitation was well conducted through the longitudinal muscle layer but such well propagation was not seen in the circular one usually. No synchronization between bursts from longitudinal and circular muscle layers was detected in the circular strip. However, the burst interaction between the layers was recorded in the L-shaped strip. The synchronization between the burst activities in the circular muscle layer was also observed in the L-shaped strip.

3. The sensitivities of longitudinal and circular muscles to oxyocin were different. Circular muscle was more insensitive to oxytocin.

4. It is concluded from the present experiment that the layers are coupled together and the excitation of one muscle layer is likely propagated into the other muscle layer by some interactions.

REFERENCES

1) Kottegoda, S.R.: An analysis of possible nervous mechanisms involved in the peristaltic reflex. *J. Physiol.*, 200 : 687-712, 1969.
2) Kobayashi, M. Nagai, T. and Prosser, C.L.: Electrical interaction between muscle layers of cat intestine. *Am. J. Physiol.*, 211 : 1281-1291, 1966.
3) Clegg, P.C.: The effect of adrenergic blocking agents on the guinea-pig uterus in vitro, and a study of the histology of the intrinsic myometrial nerves. *J. Physiol.*, 169 : 73-90, 1963.
4) Woodbury, J.W. and Brady, A.J.: Intracellular recordings from moving tissues with a flexibly mounted microelectrode. *Science*, 123 : 100-101, 1956.

5) Osa, T.: An interaction between the electrical activities of longitudinal and circular smooth muscles of pregnant mouse uterus. *Jap. J. Physiol.*, 24 : 189-203, 1974.
6) Christensen, J., Caprilli, R. and Lund, G.F.: Electrical slow waves in circular muscle of cat colon. *Am. J. Physiol.*, 217 : 771-776, 1969.
7) Bortoff, A. and Sachs, F.: Electrotonic spread of slow waves in circular muscle of small intestine. *Am. J. Physiol.*, 218 : 576-581, 1970.
8) Wood, J. D. and Perkins, W.E.: Mechanical interaction between longitudinal and circular axes of the small intestine. *Am. J. Physiol.*, 218 : 762-768, 1970.
9) McKirdy, H.C.: Functional relationship of longitudinal and circular layers of the muscularis externa of the rabbit large intestine. *J. Physiol.*, 227 : 830-853, 1972.
10) Brownlee, G. and Harry, J.: Some pharmacological properties of the circular and longitudinal muscle strips from the guinea-pig isolated ileum. *Brit. J. Pharmacol.*, 21 : 544-554, 1963.