Relationship between Action Potential and Phasic Contraction in Ureter Strip by Electrical Stimulation

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In taenia coli (Bülbring, 1955) and uterus smooth muscle (Landa et al, 1959), tension increased with repetitive spike discharges usually. Ureter smooth muscle was different from these smooth muscles in the relation between action potential and contraction. All or none response to the electrical stimulation is known to occur in ureter smooth muscle through the studies of Bozler (1942) and Prosser et al (1955). Furthermore the configuration of action potential in ureter was different from taenia coli in normal condition, that is the plateau type and the oscillatory spikes superimposed on the plateau phase. These results were recognized by intracellular recordings (Irisawa et al, 1962; 1963, Kuriyama et al, 1967). In this experiment the relationship between action potential and phasic contraction and the effect of repetitive stimuli on contraction of guinea pig ureter have been examined.

METHODS

In this experiment the electrical and mechanical activity of ureter of guinea-pig were recorded by the sucrose-gap method and the mechano-electronic transducer, using a RCA 5734 valve, simultaneously. The gap apparatus is due to Stämpfli (1954) and the isolated ureter of guinea-pig, about 3 cm in length, was mounted in the apparatus. The renal end of strip was connected to the transducer and the other end was fixed. The normal Krebs's solution used in the experiment contained (mM) : NaCl 120.7 ; KCl 5.9 ; $CaCl_2$ 2.5 ; $MgCl_2$ 1.2 ; $NaHCO_3$ 15.5 ; NaH_2PO_4 1.2 and dextrose 11.5 and was oxygenated. The sucrose-solution used contained 10 % sucrose (w/v) in distilled water. The solution flowing through the inactive side of the gap was at low temperature. The normal solution, at 37°C, flowed through the active side of the apparatus, continuously at the rate of 8-10 ml/min. Changes of membrane potential were measured from a pair of chloride silver wires, embedded in 3 % agar gel containing 3 M-KCl. Both the electrical and mechanical changes were recorded photographically from the oscilloscope (Tektronix, 502) and with the penrecorder (Nihon Koden, WI 180). The electrical stimulation was supplied through the stimulator (Grass, S-4) with the isolation unit (Grass, SIU-4B).

RESULTS AND DISCUSSION

I. Action potential and phasic contraction

Fig. 1 shows the action potential and phasic contraction of ureter smooth muscle evoked by electrical stimulation. When the stimulations were supramaximal there were no responses even gradually (Fig. 1 a, b and c). The intensity of these stimuli were from 1 V to 2 V, steps of 0.5 V and 10 msec in duration. When the strength of 2.5 V or more were applied, the action potential and phasic contraction occurred as shown in Fig. 1, d-g. These observations show obviously that the action potential and phasic contraction of ureter occur in "all or none"relation.

Usually ureter smooth muscle responsed with one action potential and one phasic contraction to each effective stimulation as shown in Fig. 1 e. This property was very different from other smooth muscles, taenia coli or uterine smooth muscle. In taenia coli smooth muscle, as reported previously, a burst type action potential is correspond to a tonic contraction. In this experiment, however, this preparation exhibited sometimes one responsed action potential to an effective stimulation and the second action potential succeeded it, the latter occurred spontaneously. The interval between these two action potentials was 2. 7 sec. In this period, ureteral smooth muscle may be in a refractory period. When the responsed action potential occurred by the effective stimulations from 2.5 V to 4 V, the maximum amplitude of action potential changed slightly and ranged from 19.6 mV to 20.2 mV.

The configuration and duration of action potential of ureter obtained by the sucrose-gap method was similar to that obtained by the intracellular recording. The duration of evoked action potential was about 0.8–1.0 sec (Table I. (1)) with the sucrose-gap method, while that was 1.5–1.7 sec with the intracellular recording (Irisawa et al, 1963). Furthermore, compared the oscillatory spikes on the plateau in both recordings, Irisawa et al (1963) reported that eight distinct oscillations superimposed on the plateau in typical example of the intracellular recording. By the sucrose-gap method, the oscillatory spikes on the plateau of action potential were from four to six (Table I. (6)).

The phasic contraction due to the second spontaneous action potential was additional. This phenomenon was resemble to the summation of contraction in skeletal muscle. Fig. 1 d shows such summation of phasic contractions.

Fig. 2 shows the effect of paired stimuli on ureter preparation. These results were taken from the continuous records. The intensity and duration of each stimulation were 4 V and 30 msec and the interval between these two stimuli varied from 2 sec to 5 sec. In the case of interval of 2 sec, the second stimulation could not triggered an action potential or a slow depolarization of membrane. By the intracellular recording (Irisawa et al 1963), similar result was obtained that no response was found when the second stimulus was applied at the early repolarization phase of first action potential.

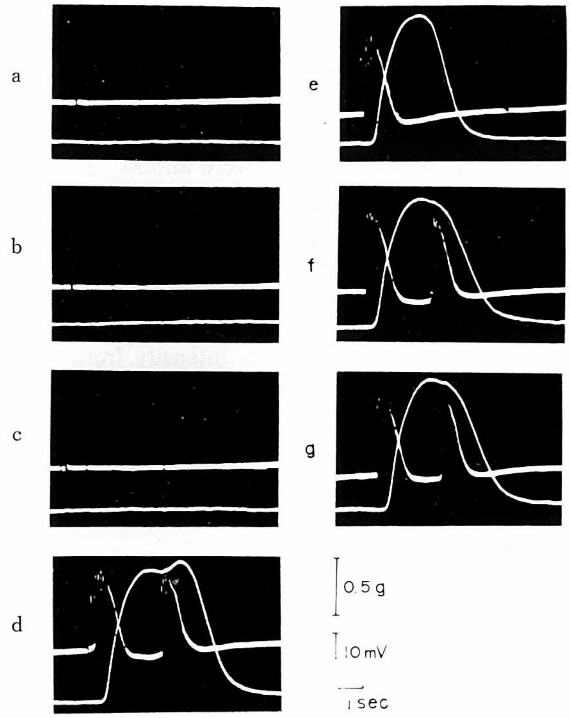

Fig. 1. Action potentials (upper) and phasic contractions (lower) of guinea pig ureter. Action potentials were elicited in response to the electrical stimulation with various intensity. a, 1V ; b, 1.5V ; c, 2V ; d, 2.5V ; e, 3V ; f, 3.5V and g, 4V (duration 10msec, constant). The second spontaneous action potential was seen in d, f and g.

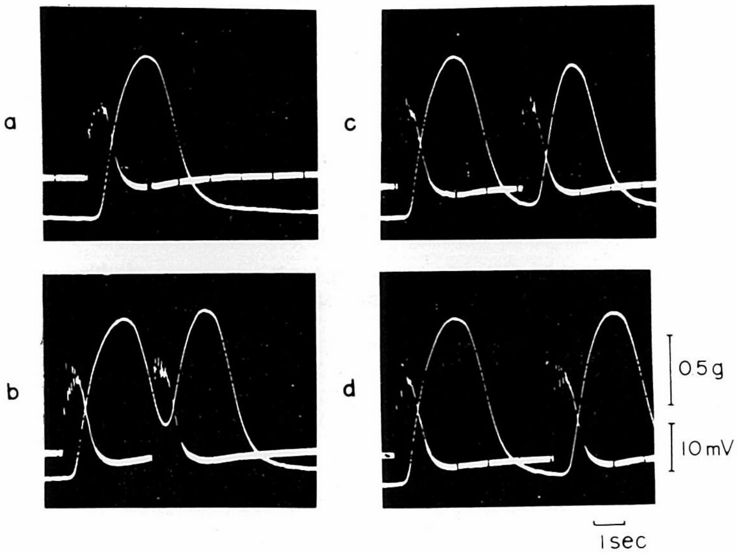

Fig. 2. Action potentials (upper) and phasic contractions (lower) of guinea pig ureter. Action potentials were elicited in response to double stimulations with various intervals. a, 2sec ; b, 3sec ; c, 4sec ; d, 5sec (intensity 4V ; duration 30msec).

More longer interval stimulations could trigger the second action potentials and phasic contractions. These results suggest that the refractory period of ureter smooth muscle is in the time between 2 second and 3 second. Irisawa et al (1963) reported that the refractory period of ureter of cat was found to be more than 1.3 sec. The second action potentials were almost same in configuration but the duration was reduced slightly. The process of second phasic contraction was different slightly from the first as shown in Fig. 2.

II. Relationship between action potential and contraction.

The parameters of action potential evoked by the effective stimulation which had the constant duration of 10 msec and the intensity from 3 V to 10 V, interval of 1 min, were measured. As shown in Fig. 3, the parameters were as follows; 1. duration of action potential, 2. amplitude of oscillatory spike, 3. amplitude of plateau, 4. plateau time, 5. maximum amplitude of oscillatory spike, 6. number of oscillatory spikes, 7. mean rate of rise of plateau (= amplitude of plateau/plateau time) and 8. mean rate of fall of plateau (= amplitude of plateau/(duration of action potential−plateau time)). The measured results were shown in Table I.

Table I. Changes in parameters of action potential with various intensity of stimulations

Intensity of stimulation (V)	(1) Duration of action potential (sec)	(2) Amplitude of action potential (mV)	(3) Amplitude of plateau (mV)
3	0.92	16.8	8.9
4	0.92	17.5	10.4
5	0.92	17.9	10.4
6	0.84	17.5	10.4
7	0.82	15.7	10.7
8	0.79	16.8	14.3
9	0.79	15.7	13.6
10	0.82	15.7	13.7

(4) Plateau time (sec)	(5) Maximum amplitude of oscillatory spike (mV)	(6) Number of oscillatory spikes
0.45	8.6	6
0.37	8.9	6
0.34	10.0	6
0.26	8.9	6
0.34	8.6	4
0.24	3.9	4
0.21	2.5	4
0.21	3.9	4

(7) Mean rate of rise of plateau (mV/sec)	(8) Mean rate of fall of plateau (mV/sec)
19.8	18.9
28.1	18.9
30.6	17.9
40.0	17.9
31.5	22.3
59.6	26.0
64.8	23.5
64.8	22.3

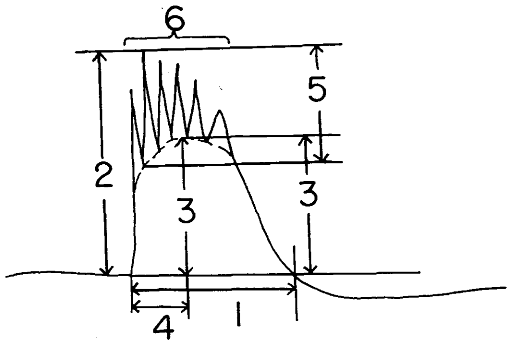

Fig. 3. Parameters in the configuration of action potential.

With increasing the intensity of stimulation, the following tendency was observed that decreasing the duration of action potential, amplitude of action potential, plateau time, maximum amplitude and number of oscillatory spikes, and increasing the amplitude of plateau, mean rate of rise of plateau and mean rate of fall of plateau. These results show that the configuration of action potential obtained by the sucrose-gap method was changed with the intensity of external electrical stimulation. The changes in configuration of action potential which evoked by the relative weak stimulations (Fig. 4 A, B) and by the stronger stimuli (C, D) were shown in Fig. 4.

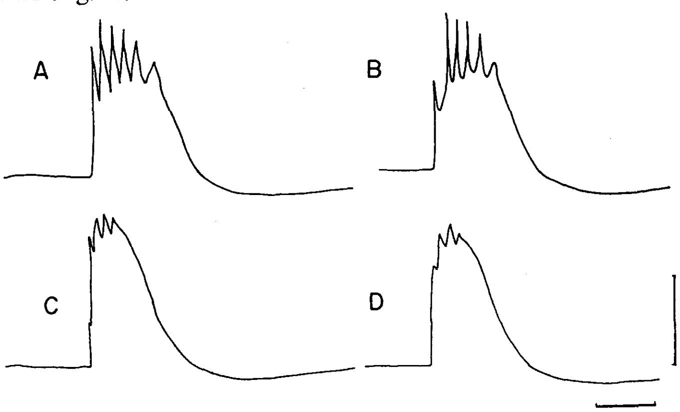

Fig. 4. Responsed action potentials of guinea pig ureter. Intensity of electrical stimulation ; A, 4V ; B, 6V ; C, 8V and D, 10V (duration 10msec). Voltage calibration 10mV and time 0.5sec.

Above results suggest that the plateau component of action potential was increased and the spike component was masked by the plateau with the increasing of intensity of external stimulation.

The parameters of phasic contraction were measured as shown in Fig. 5. The measured parameters were as follows; 1. maximum tension, 2. latency of contraction, 3. contraction time, 4. half duration, 5. relaxation time, 6. mean rate of rise of contraction (= maximum tension/contraction time) and 7. mean rate of fall of contraction (= T $(1-1/e)$/relaxation time). The measured and calculated results were shown in Table II.

Table II. Changes in parameters of phasic contraction with various intensity of stimulations

Stimulation intensity (V)	(1) Maximum tension (g)	(2) Latency of contraction (sec)	(3) Contraction time (sec)	(4) Half duration (sec)	(5) Relaxation time (sec)	(6) Mean rate of rise of contraction (g/sec)	(7) Mean rate of fall of contraction (g/sec)
3	1.07	0.45	1.50	1.84	1.65	0.71	0.65
4	1.12	0.26	1.53	1.92	1.15	0.73	0.61
5	1.13	0.23	1.53	1.89	1.10	0.74	0.65
6	1.09	0.27	1.47	1.87	1.08	0.74	0.64
7	1.05	0.26	1.45	1.84	1.08	0.72	0.62
8	1.21	0.23	1.32	1.79	1.08	0.92	0.71
9	1.22	0.21	1.42	1.71	0.92	0.86	0.84
10	1.24	0.24	1.39	1.71	0.95	0.89	0.82

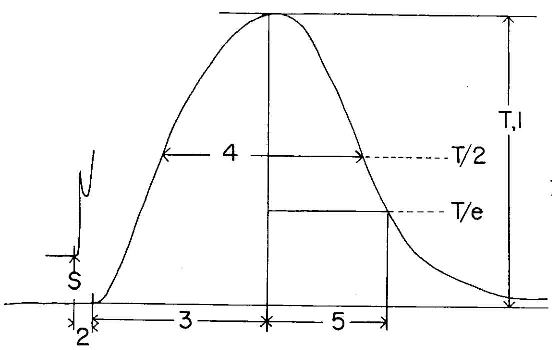

Fig. 5. Parmeters in the configuration of phasic contraction.

With increasing the intensity of stimulation, the following tendency was observed that increasing the maximum tension, mean rate of rise of contraction and mean rate of fall of contraction, and decreasing the contraction time, half duration and relaxation time.

The correlation coefficient and regression line between the mean rate of rise of plateau of action potential and that of contraction were calculated. The correlation coefficient was 0.942 and the regression line of the mean rate of rise of plateau (x) on the mean rate of rise of contraction (y) was $y = 0.004x + 0.62$. While the correlation coefficient between the mean rate of fall of plateau and that of contraction was 0.750, and the regression line of the mean rate of fall of plateau (x) on that of contraction (y) was $y = 0.022x + 0.226$. These relations were shown in Fig. 6 A and B.

These calculated results suggest that the amplitude of plateau and the rate of rise of plateau were correlated with the development of contraction closely.

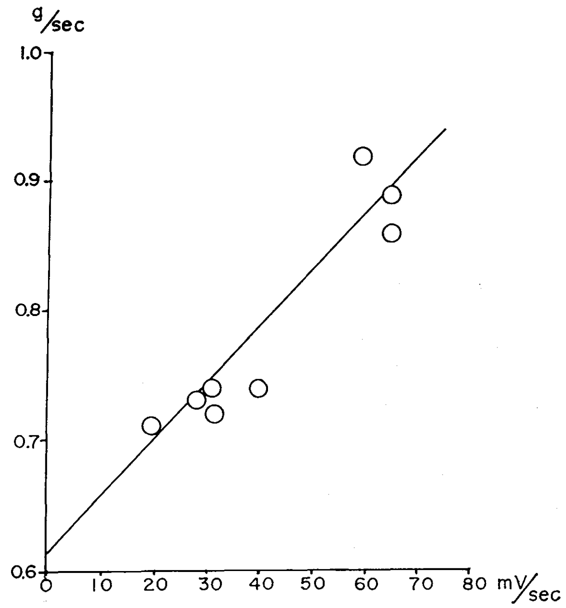

Fig. 6 A. Correlation between the mean rate of rise of plateau (Table I, (7)) and that of contraction (Table II, (6)). The solid line shows the regression line.

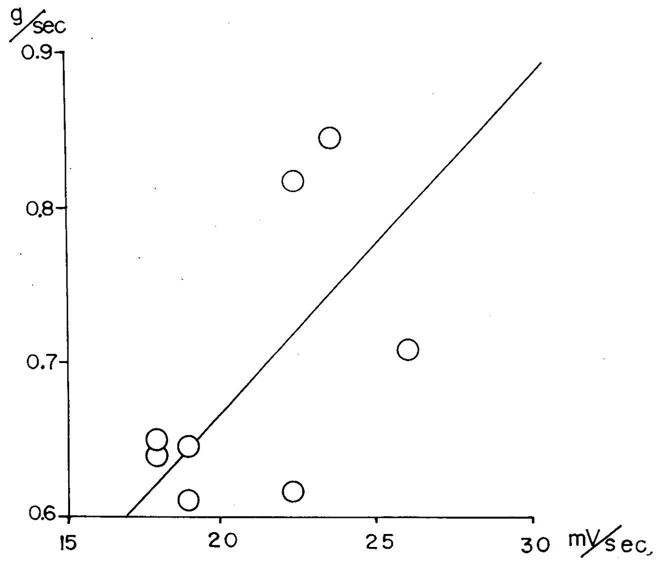

Fig. 6. B. Correlation between the mean rate of fall of plateau (Table I, (8)) and that of contraction (Table II, (7)). The solid line shows the regression line.

III. Effect of repetitive stimuli

The stimulation intensity (2.5 V; 10 msec) was constant and the frequencies of stimulation were altered as follows; A, 1 impulse/5 sec, B, 1 impulse/2 sec and C, 3 impulses/1 sec.

The responsibility of ureter changed with the stimulating period and with the frequencies of stimulation. The repetitive stimulation was continued during 3 min. Measurements were made over each 1 min period. The result of measurement was shown in Table III. In this table the parameters were calculated as follows; mean response percentage = (number of contraction in 1 min/number of stimulation in 1 min) × 100 (%) and mean response interval = 60 sec/number of contraction in 1 min (sec).

Table III. Changes on responsibility of ureter muscles in various frequency of stimulations

	Case A		
	0-60″	60-120″	120-180″
Number of stimulation	12	12	12
Number of response	11	9	6
Mean response percentage	91.7	75.0	50.0
Mean response interval (sec)	5.5	6.7	10.0

Case B			Case C		
0-60″	60-120″	120-180″	0-60″	60-120″	120-180″
30	30	30	180	180	180
6	5	4	7	4	4
20.0	16.7	13.3	3.9	3.9	2.2
10.0	12.0	15.0	8.6	15.0	15.0

As shown in Table III, it was found that the mean response percentage decreased gradually and the mean response interval prolonged with the stimulating period in each case. Thus the responsibility was decreased gradually but a contraction which was elicited to response to a stimulation had an preceded action potential always. Fig. 7 shows the contraction which were elicited by the stimulation with various frequencies. The obtained result suggests that the absolute refractory period and/or the inactivation of membrane due to the repetitive stimulations are prolonged.

The prolongation of response interval in case B and C were shown in Fig. 8.

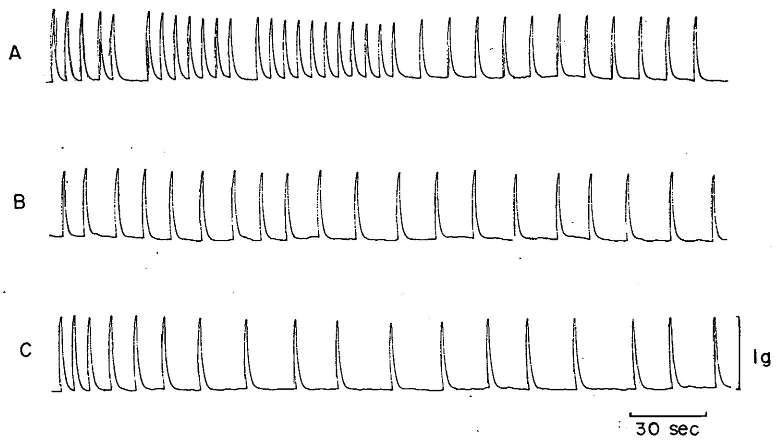

Fig. 7. Contractions of guinea pig ureter. Contractions were elicited in response to the electrical stimulation with various frequency. Frequency of stimulation; A, 1 impulse/5sec; B, 1 impulse/2sec and C, 3 impulses/1sec. Stimulation; 2.5V; 10msec.

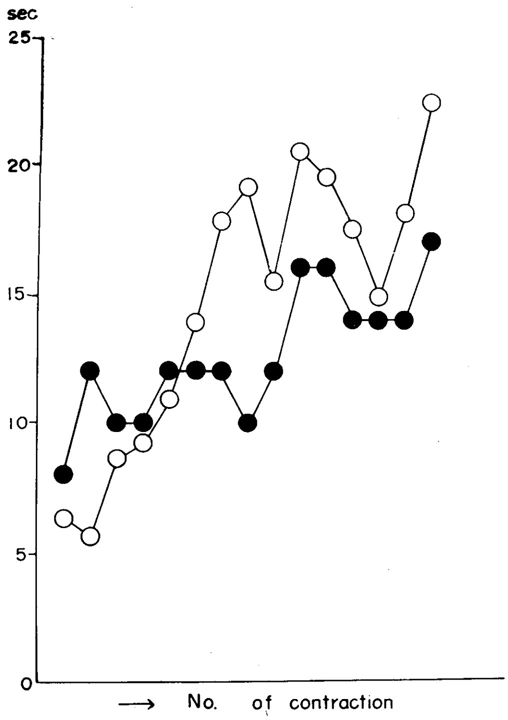

Fig. 8. Prolongation of response interval to stimulations of different frequencies. Case B, ○; Case C, ●.

Case A. When the frequency of stimulation was 1/5sec, the contraction occurred by each stimulus during first 90 sec. After this period, the contraction occurred once in two stimuli. The mean response percentage decreased gradually with the number of stimulation. When the tension of first contraction was as 100 %, after 90 sec it decreased to 76 %. However, after that, it recovered slightly, this may due to the prolongation of response interval.

Case B. The frequency of stimulus was 1/2 sec. In this case the mean response percentage decreased initially as shown in Table III, it decreased to 20 % during first 1 min and decreased furthermore to 13.3 % later period. In this case, however, the tension of contraction increased than that of the first contraction.

Case C. The frequency was very high, 3/sec. Even by this frequency of stimulation the preparation did not occur a tetanization. Namely the phasic contraction occurred only when the action potential induced by the effective stimulation. The mean response percentage was very low, it was 3.9 % during the first 1 min, and the tension of contractions decreased gradually as same as case A.

SUMMARY

1. The relationship between action potential and phasic contraction and the effect of repetitive stimuli on contraction of ureter strip have been examined by means of the sucrose-gap method and the mechano-electronic transducer.
2. The action potential and phasic contraction of ureter were in the manner of all-or-none response and its refractory period was in the time between 2 second and 3 second.
3. When increased the intensity of stimulation, the configuration of action potential and phasic contraction was changed slightly.
4. Repetitive stimuli affected on the responsibility of ureter strip. In general, the mean response percentage decreased steeply with a high frequent stimulation.

REFERENCES

1) Irisawa, H. and Kobayashi, M.: Effects of repetitive stimuli and temperature on ureter action potentials. *Jap. J. Physiol.*, **13**: 421-430, 1963.
2) Bozler, E.: The action potentials accompanying conducted responses in visceral smooth muscles. *Amer. J. Physiol.*, **136**: 553-560, 1942.
3) Bulbring, E.: Correlation between membrane potential, spike discharge and tension in smooth muscle. *J. Physiol.*, **128**: 200-221, 1955.
4) Ichikawa, S. and Ikeda, O.: Recovery curve and conduction of action potentials in the ureter of guinea-pig., *Jap. J. Physiol.* **10**: 1-12, 1960.
5) Irisawa, H. and Kobayashi, M.: Intracellular action potentials of the guinea-pig ureter. *Proc. Jap. Acad.*, **38**: 171-175, 1962.
6) Landa, J. F., West, T. C. and Thiersch, J. B.: Relationships between contraction and membrane electrical activity in the isolated uterus of the pregnant rat. *Amer. J. Physiol.*, **196**: 905-909, 1959.
7) Burnstock, G. and Straub, R. K.: A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. *J. Physiol.*, **140** 156-167, 1958.
8) Stämpfli, R.: A new method for measuring membrane potentials with external electrodes. *Experientia*, **10**: 508-509, 1954.
9) Prosser, C., Smith, C. E. and Melton, C. E.: Conduction of action potentials in the ureter of the rat. *Amer. J. Physiol.*, **181**: 651-660, 1955.
10) Cuthbert, A. W.: The relation between response and the interval between stimuli of the isolated guinea-pig ureter. *J. Physiol.*, **180**: 225-238, 1965.
11) Bennett, M. R., Burnstock, G., Holman, M. E. and Walker, J. W.: The effect of Ca^{+2} on plateau-type action potentials in smooth muscle. *J. Physiol.*, **161**: 47-48P, 1962.
12) Kuriyama, H., Osa, T. and Toida, N.: Membrane potentials of the smooth muscle of guinea-pig ureter. *J. Physiol.*, **191**: 225-238, 1967.