Studies Concerning the Action of Trivalent Cations on the Mechanical Response of Guinea-pig Ureter

Hiromichi OHKAWA

Department of Physiology, (Director: Prof. H. Niu), Yamaguchi University School of Medicine, Ube, Japan

(Received June 5, 1974)

Lanthanum (La^{+++}) appears to have a specific effect on Ca flux[1,2] and to related an inhibition of contrcation on smooth muscle,[3-9] cardiac muscle [1,10,11] and skeletal muscle[12,13]. The electrical activity of membrane is also affected by La^{+++} treatment[1,14]. At the present, the mechanism of inhibitory action of La^{+++} on the contractile system is considered as follows; (1) La^{+++} affects superficial calcium sites more than other cellular Ca^{++} stores, (2) Ca flux is modified by the binding of La^{+++} to anionic sites. Althought La^{+++} could not penetrate into the cell[3,5,9,14,15], a direct action of La^{+++} to the contractile system, bound calcium and/or intracellular Ca^{++} can not be exclude. Since the site of action of La^{+++} has been postulated to be at superficial cellular locations, an attempt has been made to examine whether various trivalent cations such as holmium (Ho^{+++}), samarium (Sm^{+++}) and yttrium (Y^{+++}) act at the cell surface and what parts of Ca^{++} are affected by the trivalent cation treatment with various ionic environments.

METHODS

Guinea-pigs of either sex, weighing between 200g and 500g, were used. The animals were stunned and bled. A suitable length, 2–2.5 cm, of ureter was removed. The tubular segment of ureter was suspended in an organ bath containing 300ml of the Tris-solution bubbled with 95% O_2 and 5% CO_2. The temperature was maintained at between 36 and 37°C with a heated water-bath. The free end of preparation was tied to a mechano-electronic transducer. Paired silver ring was used for electrical stimulation. Above the threshold, single stimulation can produce a phasic contraction. In these experiments, 10–30V, 300 msec in duration of stimulation was used. The interval was 20–30 sec.

The bathing solution employed in these experiments was idential to the Tris-buffer solution used by Goodman et al[7]. This solution had the following composition: NaCl, 154mM; KCl, 5.4mM; $CaCl_2$, 1.5mM; glucose, 6mM and tris (hydroxymethyl) amino-methane, 6 mM. Solutions were

prepared in distilled water and adjusted with a pH meter to pH 7.60 by addition of small volumes of 4N HCl. The following drugs were used for trivalent cations; holmium chloride (Ho^{+++}), lanthanum chloride (La^{+++}), samarium chloride (Sm^{+++}) and yttrium chloride (Y^{+++}). These agents were pipetted from concentrated stock solutions directly into the bathing media to give the final concentration desired.

RESULTS

When electrical stimulation interval was 20 sec or more, the ureter preparation produced a phasic contraction by single stimulation. The tension development was nearly equal in successive contractions. In some preparations, the spontaneous phasic contraction was generated in normal solution. However, in this experiment, the responsed regular contraction was used as a control. After the regular phasic contraction was obtained, drugs were added into the organ bath.

Effects of trivalent cations

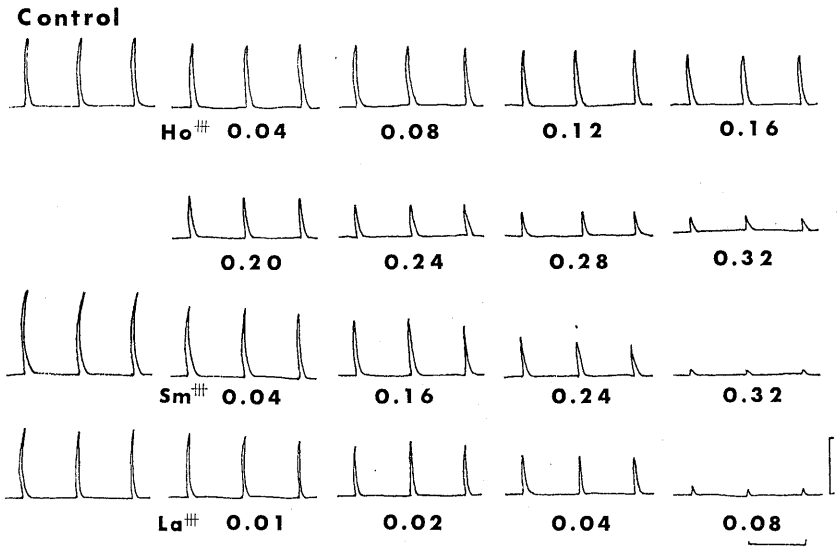

Fig 1. Effects of various trivalent cations on the phasic contraction of ureteral smooth muscle.
Left; Control responses produced by single electrical stimulations at interval of 30sec. Right (four rows); Responses in the various concentrations of trivalent cations. Numbers indicate the concentrations of trivalent cations in mM. The responses in cations were obtained after 5-6min incubation. Calibration; 30 sec and 0.5g.

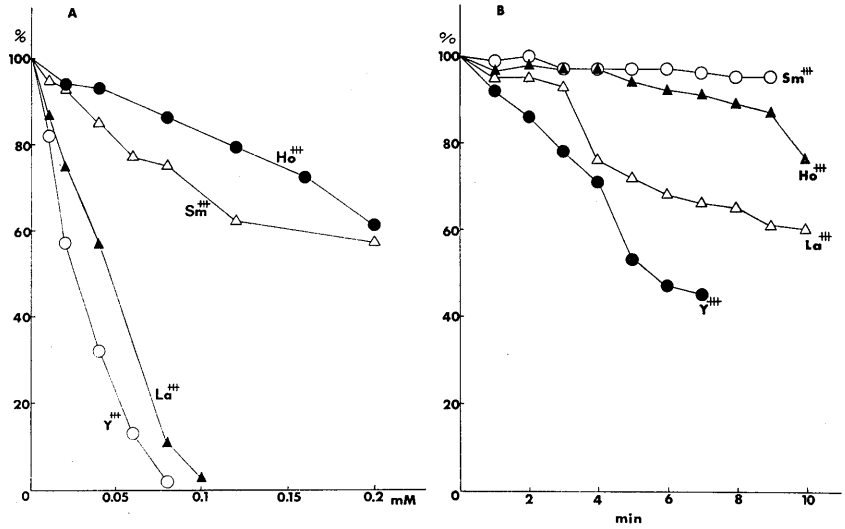

Fig 2. A; Inhibition by trivalent cations on the phasic contraction of ureteral smooth muscle. The values were obtained at 10min incubation with various trivalent cations. The magnitude of phasic tension in normal solution was used as 100%. B; Time courses of the inhibitory responses in various trivalent cations. Concentrations of trivalent cations were 0.01mM.

The tension development was gradually reduced in the solution of various trivalent cations (Fig. 1) and it took long time to reach a certain equilibrated level in tension. Usually the effect of trivalent cations on the phasic contraction was measured at 10 min after immersion with a trivalent cation. All species of trivalent cations exhibited an inhibitory effect on the tension development. However the degree of inhibitory effect was different with concentrations and cation species. As shown in Fig. 2, La^{+++} and Y^{+++} showed strong inhibitory effect while Sm^{+++} and Ho^{+++} showed weak.

Effects of TEA and trivalent cations

Tetraethylammonium chloride (TEA) potentiated the tension of phasic contraction. The does-response curve of TEA during trivalent cation treatment was shown in Fig. 3. After incubation with trivalent cation solutions during 10 min, TEA (0.1–3.2mM) was added into the organ bath. The tension was still potentiated in Sm^{+++} (0.08mM) and increased with the TEA concentrations. After pre-treatment with Y^{+++} (0.01mM) or La^{+++} (0.02 mM), the magnitude of phasic contraction was smaller than normal value but the contraction was potentiated with TEA concentrations. For comparison of the action of trivalent cations, same concentration (0.014 mM) of these cations and constant TEA (3.2mM) were applied to the same preparation. The inhibitory action for TEA-induced potentiation was separated

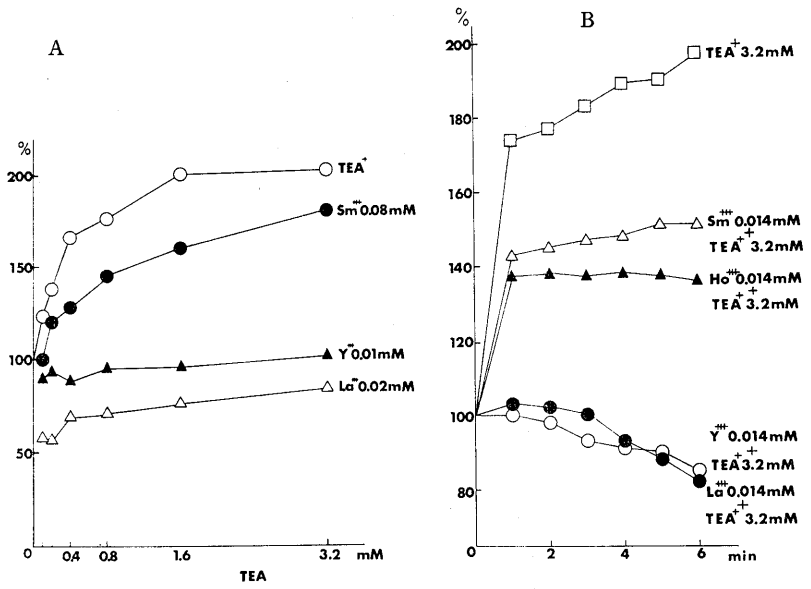

Fig 3. Effects of trivalent cations and TEA on the phasic contraction. A; Relations between TEA ranged from 0.01 mM to 3.2mM and Sm^{+++}, Y^{+++} and La^{+++}. B; Time courses of the effects of trivalent cations and TEA on the phasic contraction. TEA concentrations was 3.2mM and trivalent cations 0.014 mM. These experiments were carried out from same preparation.

into two groups, i. e., La^{+++} and Y^{+++} belonged to strong inhibition group and weak group contained Ho^{+++} and Sm^{+++}.

Effects of Ba^{++} and trivalent cations

Barium ion (Ba^{++}) also potentiated the phasic contraction. High concentrations of Ba^{++} (above 0.5mM) produced considerable spontaneous activity having a high frequency of phasic contraction. Even in low concentrations of Ba^{++} (below 0.5mM), the magnitude of tension was increased with Ba^{++} concentrations and exposure period. These potentiations were also inhibited by treatment with various trivalent cations. In these experments, it was found that the inhibitory action of cations was grouped into two types, that is, Y^{+++}, La^{+++} group and Sm^{+++}, Ho^{+++} group.

Effects of Sr^{++} and trivalent cations

The phasic contraction was not potentiated by strontium (Sr^{++}) up to 3mM. However interesting result on the inhibitory action of trivalent cations with Sr^{++} was obtained. The inhibitory effect of Sm^{+++} (0.04mM) or La^{+++} (0.04mM) was increased when Sr^{++} was present in the media. Especially, the effect of La^{+++} was potentiated when 3mM Sr^{++} was present. After 2–3min in such condition, the response was abolished abruptly.

Effect of Mn++ and trivalent cations

Mn++ inhibited the phasic contraction of preparation. The inhibition which was observed in the Mn++ solution above 0.04mM, was depended on the external Mn++ concentrations. After 10 min in 0.12 mM of Mn++ solution, the responses were not generated. In comparison of the inhibitory effect of Mn++ with trivalent cations, the order of inhibition was Mn++> La+++> Sm+++, in the same concentration (0.08mM). Fig. 4 shows the time courses of inhibition by Mn++and trivalent cationts.

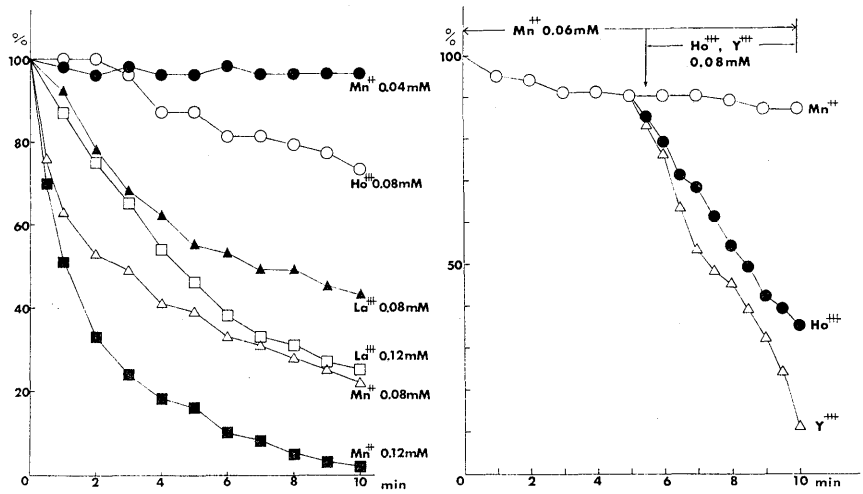

Fig 4. Comparison of Mn++ and trivalent cations on the phasic contractions. Left; Time courses of the effects of Mn++, La+++ and Ho+++. Right; Effects of addition of Ho+++ and Y+++ during Mn++ solution.

Effects of Ca++ and trivalent cations

High calcium solutions (3–9mM) produced variable changes in tension development. The magnitude of the tension was gradually increased in double calcium concentration (3mM). After 10 min in this solution, the tension was about 150% of normal. The phasic contraction was reduced in higher Ca++ concentrations (4.5–9mM). In the concentration of 9mM Ca++, the phasic contraction was reduced to approximately 70% of normal during 10 min incubation. After 10 min incubation with 3mM Ca++ solution, trivalent cations was added into the external solution. The phasic contraction was gradually reduced by such treatment. However the great reduction of tension was observed by Y+++ addition (0.02mM).

When Ca++ was removed from the external solution, the phasic contraction was reduced immediately. No responses were usually observed in

Ca-free solution after 10 min. The decrease of tension was almost linear with the period in Ca-free media. Ba^{++} and Sr^{++} recovered the response while TEA (3.2–6.4mM) could not. The contractile activity was disappeared more rapidly in Ca-free solution including trivalent cations. La^{+++} group indicated the rapid reduction. After abolition of response, Ba^{++} (0.3mM) generated it in Ca-free, Ho^{+++} (0.3mM) solution. However, Ba^{++} (0.3mM) could not recover the response in Ca-free, La^{+++} (0.3mM) solution (Fig. 5).

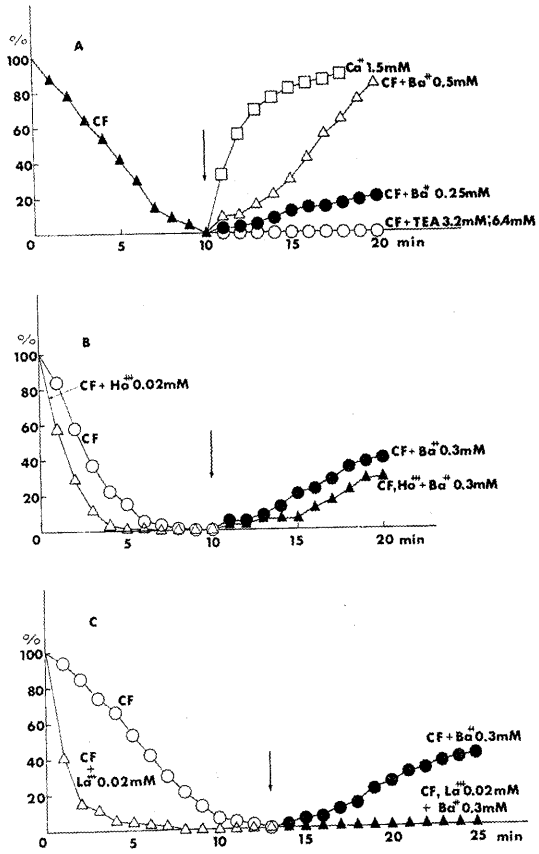

Fig 5. Effects of external Ca^{++} and trivalent cations on the phasic contraction. A; The phasic contraction was abolished in Ca-free media rapidly. Arrows indicate the second treatment with ions. Recovery was observed in Ca^{++} and Ca^{++}-free, Ba^{++} solution while it did not in Ca-free, TEA solution. B and C; After blocking the responses in Ca-free, Ca-free + Ho^{+++} or Ca-free + La^{+++} solution, Ho^{+++} and La^{+++} inhibited the recovery produced by Ba^{++}. The strong inhibitions were also observed in Ca-free + Ho^{+++} and Ca-free + La^{+++} solution.

Potassium contracture and trivalent cations

Fig. 6 shows the phasic contraction and potassium contracture produced

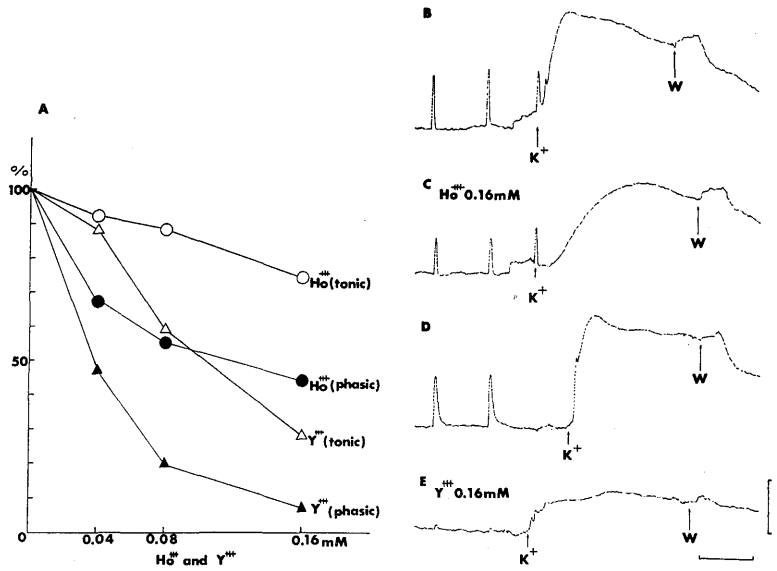

Fig 6. Relationship between potassium contracture and trivalent cations. A; Comparison of the inhibitory action of Ho^{+++} and Y^{+++} on phasic contraction and tonic component of potassium contracture. B; Control responses of phasic contraction and potassium contracture in normal Ca^{++} for C. C; Decreases in phasic and tonic contraction in Ho^{+++} 0.16mM. Note the time course of potassium contracture compared with the control. D; Control responses for E. E; Decreases in phasic and tonic contraction in Y^{+++} 0.16 mM. The phasic contraction was abolished almostly. The potassium contracture was still present but the magnitude was small.

by isotonic potassium solution. Rapid phasic contractions were observed in the intial stage of potassium contracture. Tension was increase above the phasic contraction level and reached the maximum level within 20 sec in all preparations. Then it was reduced gradually. Potassium contracture was depressed in Ho^{+++} and Y^{+++} solution. In 0.16mM of Ho^{+++} solution, the time course of tension development was very different from the control. Potassium contracture in which the phasic component was disappeared by trivalent cation was increased more slowly and reached the maximum level at about 40–60 sec. Effect of trivalent cations on the potassium contracture was summarized in Fig. 6. Both phasic and tonic (potassium contracture) ïcontraction were inhibited by external trivalent cations. In comparison of the inhibition on phasic and tonic contraction, the inhibition on phasic was stronger than that on tonic.

When the external calcium concentration was ranged from 0.2mM to 2mM, the magnitude of the potassium contracture were depended on external Ca^{++} concentrations. The relaiotnship between the external Ca^{++}

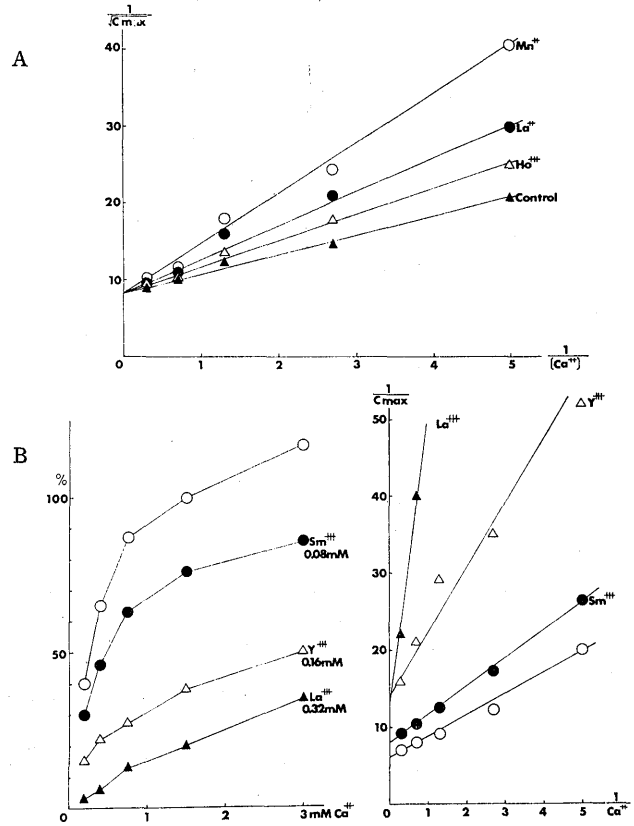

Fig 7. Relationship between external Ca^{++} and potassium contracture. Upper; Lineweaver-Bulk plot on the external Ca^{++} concentrations and the maximum responses of potassium contracture in Ho^{+++}, La^{+++} and Mn^{++} solution. The concentration of these cations was 0.04mM. Lower left; Usual plot of the relation between external Ca^{++} concentration and potssium contracture. White circles indicate the cntrol in each of Ca^{++} concentrations. Lower right; Lineweaver-Bulk plots on the data obtained from the left.

concentration and potassium contracture and that in La^{+++}, Ho^{+++} and Mn^{++} solution were shown in Fig. 7A.

Potassium contractures in normal and in trivalent cations were depended on the external Ca^{++} concentration. Fig. 7A is a Lineweaver-Bulk plot on the data showing the relationship between the reciprocals of calcium concentration and responses. In above cases, lower concentrations of trivalent cations and Mn^{++} were used. On the other hand, such linear relations were not detected when used high concentrations of trivalent cations (0.08–0.32 mM) as shown in Fig. 7B.

DISCUSSION

The phasic contraction of ureteral smooth muscle was inhibited by various trivalent cations. Observations on ileal smooth muscle[9] and on uterine smooth muscle[8] showed that, in the presence of La^{+++}, the responses of these tissues were also inhibited. In this observation, the La^{+++} and Y^{+++} group showed stronger inhibitory action than Ho^{+++} and Sm^{+++} group. Weiss et al[9] speculated that La^{+++} was characterized as an ion capable of displacing Ca^{++} from surface sites in longitudinal smooth muscle; La^{+++} remains at these sites and exerts a stabilizing action which prevents inward release of Ca^{++}. The affinity to the anionic sites had the order of $La^{+++} > Mn^{++} > Ca^{++}$[16]. From above, Ca^{++} movement was inhibited by trivalent cations and the degree of bind ability to the sites in membrane is to be La^{+++}, $Y^{+++} > Ho^{+++}$, $Sm^{+++} > Ca^{++}$. There are no report about the action of Ho^{+++}, Sm^{+++} and Y^{+++} on the mechanical activity of other smooth muscle. In mitochondria of rat liver[2], Ho^{+++} was characterized as an ion capable of inhibiting Ca^{++} transport similar to La^{+++}.

One of the mechanisms in which the contraction was potentiated by external Ba^{++} is considered that Ba^{++} can generate the action potential as a substitute for Ca^{++}[17] and prolong the spike duration[15]. In taenia coli smooth muscle[18], the spike frequency was increased due to Ba^{++}-induced depolarization. The increase in electrical activity may trigger to increase Ca influx, bound calcium content and free Ca^{++} in the cell. Consequently the response might be increased. The addition of Ba^{++} during Ho^{+++} and Sm^{+++} treatment potentiated the response above the normal while it still depressed during Y^{+++} and La^{+++}. These results suggest that the relation between the inhibition of Ca influx due to trivalent cations and the increase in it due to Ba^{++} is competitive. Ba^{++} might be relate to tight bound calcium as discussed later.

TEA enhanced the spike amplitude of the stomach smooth muscle markedly[18]. TEA may increase the Ca influx. The addition of TEA during trivalent cation treatment produced the enhancement of response in Ho^{+++} and Sm^{+++} while no increase was observed in Y^{+++} and La^{+++}. These results also indicate that the competitive action due to TEA and trivalent cations to Ca^{++} utilization to contraction. This competition may be to Ca influx but not bound calcium stores.

Sr^{++} produced the decrease in number of repetitive spikes and the prolongation of plateau[19]. The magnitude of response was increased slightly in 3mM Sr^{++} solution. These results indicate some relationship between the action potential and contractile system. The recovery in response was obtained by addition of Sr^{++} during Ca-free solution. Interest-

ing results in which the inhibitory action of trivalent cations was potentiated due to coexistence of Sr^{++} in La^{+++} and Y^{+++}, were obtained. This effect was markedly in La^{+++} and Y^{+++}. The recovery by Sr^{++} during Ca-free solution was decreased rapidly and abolished finally with the addition of La^{+++} or Y^{+++}.

In Ca-free solution, the responses were decreased immediately but a relaxation which observed in other smooth muscle[20] was not occurred. The action potential was also abolished in initial stage of Ca-free solution[21]. When trivalent cation was present with Ca-free solution, abolishment of response was rapidly. The recovery of response was observed by the addition of Ba^{++} during Ca-free solution. The manner in which the substitution of Ba^{++} for Ca^{++}, release from a part of tight bound calcium of which Ca^{++} could not be release in Ca-free solution and direct activation of contractile system by Ba^{++} is considered. The long-plateau type of action potential of ureteral smooth muscle was recorded in Ca-free solution but contraction related with this action potential was not generated[21]. That is, the excitation-contraction coupling was dissociated in Ca-free solution because this action potential might not release Ca^{++} from the tight bound calcium stores. The recovery by Ba^{++} in Ca-free solution seems to utilize such tight bound calcium store in the membrane.

In the present experiments, stronger inhibition due to Mn^{++} than La^{+++} was observed. Similar results were obtained in the vascular smooth muscle[22]. Two different anionic sites in the vascular smooth muscle membrane were postulated[22]. One of two sites relates to the generation of action potential and contraction, another relates the contraction only; Mn^{++} acts to the former and La^{+++} acts the latter. This assumption, however, is not suitable in a part since trivalent cations seem to relate tight bound calcium store as shown in observation on potassium contracture. As the order of affinity to the anionic sites in membrane was $La^{+++} > Mn^{++}$[16], it is considered that the inhibitory action to Ca influx by La^{+++} was stronger than Mn^{++}. However, the converse results were obtained in this experiment. The result suggests the direct action of Mn^{++} to the contractile system and/or intracellular Ca^{++}. Mn^{++} might inhibit a release from bound calcium after penetrating into the cell[17,23].

Potassium contracture was depressed by trivalent cations. In comparison with the inhibitory action on phasic contraction and tonic contracture, the strong depression was observed in the former. Hitherto, in smooth muscle, it was postulated that the phasic component which indicates the initial contracture is depend on the external calcium ions and the tonic component indicating the late contracture is depend on the bound calcium[17]. In the ion flux measurement, many authors[3,5,22,24] reported that the Ca

influx was increased during the phasic phase of potassium contracture. The restlts obtained suggest that trivalent cations inhibit Ca influx and the release from bound calcium store and that the inhibition to Ca influx is severely. Similar results were obtained in bovine facial artery[22].

The time course of the depressed potassium contracture was different markedly. The phasic component of contracture was disappeared and the slow increase in tonic component was observed. These changes might be due to the inhibition of Ca influx and the slow release from the bound calcium. It is postulated that La^{+++} could not penetrate to intracellular space [3,5,13,14,15]. However, the penetration of some parts of trivalent cations and the inhibition of release from bound calcium store can not be exclude.

Potassium contracture was depended on the external calcium. The relationship in the Lineweaver-Bulk plot was linear. The relationships in trivalent cations and Mn^{++} treatment were also nearly linear and lines have a same cross point on the response axis. These results suggest that the utilized calcium ions for potassium contracture originate from the same calcium store and Mn^{++} and trivalent cations affect the calcium store.

It seems appropriate to conclude from the above that trivalent cations act the superficial calcium sites and inhibit Ca^{++} movement. Tight bound calcium might be also affected by these cations. Consequently the mechanical response of ureteral smooth muscle was inhibited by these cations.

SUMMARY

1. Effects of various trivalent cations on the mechanical response of smooth muscle of guinea-pig ureter were examined.

2. Responses were inhibited by trivalent cations in low concentrations. The order of inhibition was La^{+++}, $Y^{+++} > Ho^{+++}$, Sm^{+++}.

3. Potentiation in tension development due to TEA and Ba^{++} was depressed by used these cations. Sr^{++} did not increase the response in normal solution but it was recovered by the addition of Sr^{++} in Ca-free solution. The recovery by Sr^{++} also inhibited by used cations.

4. The inhibition of response was observed in Mn^{++} solution. Effect of Mn^{++} on the mechanical response was stronger than La^{+++}.

5. In Ca-free solution, recovery due to the addition of Ba^{++} was also inhibited by trivalent cations having the order of $La^{+++} > Ho^{+++}$. La^{+++} failed the Ba^{++}-induced recovery.

6. Potassium contracture was depended on the external calcium concentration. Lineweaver-Bulk plot on relationship between Ca^{++} concentration

and potassium contracture in trivalent cations and Mn^{++} indicated linear. These results indicate the competitive action to the same calcium store.

7. The action of trivalent cations to superficial calcium sites, bound calcium and Ca^{++} movement for contraction was discussed.

REFERENCES

1) Sanborn, W. G. & Langer, G. A.: Specific uncoupling of excitation and contraction in mammalian cardiac tissue by lanthanum kinetic studies. *J. Gen. Physiol.*, 56 : 191-217, 1970.
2) Mela, L.: Inhibition and activation of calcium transport in mitochondria. Effect of lanthanides and local anesthetic drugs. *Biochemistry*, 8 : 2481-2486, 1969.
3) Van Breemen, C., Farinas, B. R., Gerba, P. & McNaughton, E. D.: Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. *Circ. Res.*, 30: 44-54, 1972.
4) Freeman, D. J. & Daniel, E. E.: Calcium movement in vascular smooth muscle and its detection using lanthanum as a tool. *Can. J. Pharmacol.*, 51: 900-913, 1973.
5) Van Breemen, C.: Blockade of membrane calicum fluxes by lanthanum in relation to vascular smooth muscle contractility. *Arch. Int. Physiol.*, 77: 710-716, 1969.
6) Uemura, K.: Ca contribution to several contractions in guinea-pig taenia coli. *Sapporo Med. J.*, 42: 284-294, 1973.
7) Goodman, F. R. & Weiss, G. B.: Effects of lanthanum on ^{45}Ca movements and on contractions induced by norepinephrine, histamine and potassium in vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, 177 : 415-425, 1971.
8) Goodman, F. R. & Weiss, G. B.: Dissociation by lanthanum of smooth muscle responses to potassium and acetylcholine. *Amer. J. Physiol.*, 220 : 759-766, 1971.
9) Weiss, G. B. & Goodman, F. R.: Effects of lanthanum on contraction, calcium distribution and ^{45}Ca movements in intestinal smooth muscle. *J. Pharmacol. Exp. Ther.*, 169 : 46-55, 1969.
10) Hodgson, B. J. & Daniel, E. E.: Studies concerning the source of calcium for contraction of rat myometrium. *Can. J. Physiol. Pharmacol.*, 51 : 914-932, 1973.
11) Entman, M. L., Hansen, J. L. & Cook, J. W.: Calcium metabolism in cardiac microsomes incubated with lanthanum ion. *Biochem. Biophys. Res. Commun.*, 35 : 258-264, 1969.
12) Weiss, G. B.: On the site of action of lanthanum in frog sartorius muscle. *J. Pharmacol. Exp. Ther.*, 174 : 517-526, 1970.
13) Weiss, G. B.: Inhibition by lanthanum of some calcium-related actions in frog rectus abdominis muscle. *J. Pharmacol.*, 185 : 551-559, 1973.
14) Takata, T., Pickard, W. F., Lettvin, J. Y. & Moore, J. W.: Ionic conductance changes in lobster axon membrane when lanthanum is substituted for calcium. *J. Gen. Physiol.*, 50 : 461-471, 1966.
15) Lehninger, A. L. & Carafoli, E.: The interaction of La with mitochondria in relation to respiration-coupled Ca transport. *Arch. Biochem. Biophys.*, 143 : 506-515, 1971.
16) Hagiwara, S. & Takahashi, K.: Surface density of calcium ions and calcium spikes in the barnacle muscle fiber membrane. *J. Gen. Physiol.*, 50 : 583-601, 1967.
17) Imai, S. & Takeda, K.: Actions of calcium and certain multivalent cations on potassium contracture of guinea- pig's taenia coli, *J. Physiol.*, 190 : 155-169, 1967.
18) Ito, Y., Kuriyama, H. & Sakamoto, S.: Effects of tetraethylammonium chlovide on the membrane activity of guinea-pig stomach smooth muscle. *J. Physiol.*, 211 : 445-460, 1970.
19) Ito, Y. & Tomita, T.: The action potential in the smooth muscle of the guinea-pig taenia coli and ureter studied by the double sucrose-gas method. *J. Gen. Physiol.*, 55 : 147-162,

1970.
20) Sunano, S. & Miyazaki, E.: The initiation of contraction by extracellular calcium in the smooth muscle or the guinea-pig taenia coli. *Experientia*, 24 : 364-365, 1968.
21) Ohkawa, H.: Plateau formation in action potential of guinea-pig ureter. *Bull. Yamaguchi Med. Sch.*, 18 : 59-68, 1971.
22) Adachi, T.: Effects of lanthanium and manganese on noradrenaline contraction, potassium contracture and Ca movements in vascular smooth muscle. *Sapporo Med J.*, 41 : 123-139, 1972.
23) Shibata, S.: Effects of Mn on ^{45}Ca content and potassium-induced contraction of the aortic strip. *Can. J. Physiol. Pharmacol.*, 47 : 827-829, 1969.
24) Briggs, A. H.: Calcium movements during potassium contracture in isolated rabbit aortic strips. *Amer. J. Physiol.*, 203 : 849-852, 1962.