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Mechanism of barium-induced contraction in the vascular smooth muscle of rabbit aorta

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1 In a solution containing 1.5 mM Ca^{2+}, cumulative application of 0.3–10.0 mM Ba^{2+} induced a concentration-dependent contraction of the rabbit aorta. This contraction was reduced by the Ca^{2+} channel inhibitors, verapamil (10^{-6} M), nifedipine (10^{-7} M) and lanthanum (2.0 mM), and was potentiated by the Ca^{2+} channel facilitator, Bay K 8644 (10^{-7} M).

2 In a Ca^{2+}-free solution containing EGTA (1.0 mM), cumulative application of Ba^{2+} still induced a concentration-dependent contraction, the maximum contractile tension of which was comparable to that in the presence of 1.5 mM Ca^{2+}.

3 The Ba^{2+}-induced contraction which was not dependent on the external Ca^{2+} was also inhibited by verapamil, nifedipine and lanthanum and was potentiated by Bay K 8644. A high concentration (65.4 mM) of K^+ potentiated this Ba^{2+}-induced contraction whereas noradrenaline (10^{-6} M) did not have such an effect.

4 In order to deplete the releasable Ca^{2+} store in the cell, the muscle strip was treated with noradrenaline (10^{-6} M) and/or caffeine (20.0 mM) in a Ca^{2+}-free solution. In such a Ca^{2+}-depleted muscle, Ba^{2+} still induced a contraction of a similar magnitude to that without such treatment. Further, the second application of Ba^{2+} in a Ca^{2+}-free solution induced a similar contraction to that induced by the first application of Ba^{2+}.

5 These results suggest that Ba^{2+} depolarizes the cell membrane and opens the voltage-dependent Ca^{2+} channels resulting in a Ca^{2+} influx in the presence of Ca^{2+}. In the absence of external Ca^{2+}, Ba^{2+} may enter the cell through the voltage-dependent Ca^{2+} channels and induce contraction without mobilizing the Ca^{2+} store which is sensitive to noradrenaline and caffeine.

Introduction

Barium ion (Ba^{2+}) has been known to induce contraction in various smooth muscle tissues such as intestine (Yukisada & Ebashi, 1961; Karaki *et al.*, 1967), uterus (Daniel, 1963) and blood vessels (Somlyo *et al.*, 1974). Ba^{2+} depolarizes the smooth muscle membrane (Suzuki *et al.*, 1964; Hotta & Tsukui, 1968; Bülbring & Tomita, 1969). In intestinal smooth muscle, Karaki *et al.* (1967, 1969) compared the contractions induced by Ba^{2+} and high concentrations of K^+ and concluded that both contractions are due to Ca^{2+} influx although higher concentrations of Ba^{2+} induce a contraction which is not dependent on the external Ca^{2+} concentrations. In the present experiments, it was found that a larger portion of the Ba^{2+}-induced contraction was not dependent on the external Ca^{2+} in the vascular smooth muscle of rabbit aorta and the mechanisms of this contraction were examined.

Some of these results have been briefly reported (Karaki *et al.*, 1986).

Methods

Tissue preparations

Male New Zealand rabbits, weighing 2.0–2.5 kg, were killed by a rapid injection of sodium pentobarbitone (25 mg kg^{-1}) and air into an ear vein. The thoracic aorta was rapidly removed, cut into a spiral strip and muscle preparations, 3–4 mm wide and 5–8 mm long, were prepared. In some experiments, the adventitial layer was separated from the media-intimal layer as described by Karaki and Urakawa (1977) in order to avoid the possible involvement of endogenous cate-

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cholamines (Karaki *et al.*, 1984). However, the characteristics of the Ba^{2+}-induced contraction in these two types of preparations did not differ and the results with these preparations are not described separately.

Solutions

Since high concentrations of Ba^{2+} produced precipitation in a bicarbonate-buffered solution, the bicarbonate-free solution of the following composition was used as normal solution (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, glucose 5.5 and tris(hydroxymethyl)aminomethane (Tris) 23.8, adjusted to pH 7.4 at 37°C with 6 N HCl. Muscle contractions induced by high concentrations of K^+ or noradrenaline in this solution did not change from those in bicarbonate-buffered solution, as reported earlier (Karaki *et al.*, 1981). High K^+ solution (65.4 mM) was made by substituting 60 mM NaCl with equimolar KCl and Ca^{2+}-free solution was made by removing $CaCl_2$ and adding 1.0 mM ethyleneglycol bis(β-aminoethylether)-N,N,N',N'-tetra acetic acid (EGTA) to the above solution. These solutions were aerated with 100% O_2.

Muscle tension

Muscle tension was recorded isometrically under a 1 g resting tension with a force-displacement transducer connected to a Nihon Kohden polygraph. $BaCl_2$ was cumulatively added to the bath. When the Ba^{2+}-induced contraction was repeated, especially in a Ca^{2+}-free solution, the relaxation induced by the wash with a Ba^{2+}-free solution gradually became less complete. It was found that washing the muscle with a high K^+, Ca^{2+}-free solution induced more complete relaxation than with a Ca^{2+}-free solution. However, the relaxation of the third or the fourth contraction was sometimes less complete even if the muscle was washed with a high K^+, Ca^{2+}-free solution. Therefore, only one or two Ba^{2+}-induced contractions were induced in one muscle strip in most of the experiments. Results were expressed in terms of actual contractile tension (g). In order to compare the contractions in different muscle strips, all the preparations were stimulated during the preincubation period with noradrenaline 10^{-6} M and the strips which developed contractile tension between 1.8 g and 2.0 g (average of 1.90 ± 0.04 g, $n = 90$) were used in the present experiments. Agents to be tested were added 10 min before the application of Ba^{2+} and the incubation with a Ca^{2+}-free solution was started at least 30 min before the application of Ba^{2+}.

Statistics

Results of the experiments are expressed as mean ± s.e.mean. Student's *t* test was used for statistical analysis of the results and a *P* value less than 0.01 was taken as significant.

Drugs and chemicals

(±)-Verapamil hydrochloride (donated by Eisai), nifedipine (donated by Pfizer), Bay K 8644 (Schramm *et al.*, 1983; donated by Miles Pharmaceuticals), (−)-noradrenaline bitartrate (Wako Pure Chemical Industries), Tris (Sigma), EGTA (Sigma) and caffeine (Wako) were used.

Results

Ba^{2+}-induced contraction in normal solution

Cumulative application of 0.1–10.0 mM Ba^{2+} induced a concentration-dependent contraction in rabbit aorta, as shown in Figure 1. The second application of Ba^{2+} induced a contraction similar to that induced by the first application of Ba^{2+}.

Pretreatment of the muscle with verapamil (10^{-6} M) shifted the concentration-response curve for Ba^{2+} to the right (Figure 1). Nifedipine, 10^{-7} M, showed a similar inhibitory effect to verapamil (10^{-6} M) (data not shown). $LaCl_3$, 2.0 mM, had a stronger inhibitory effect than verapamil 10^{-6} M (Figure 1). In contrast, Bay K 8644, 10^{-7} M, shifted the concentration-response curve for Ba^{2+} to the left and increased maximum contractile tension by 16.2%, as shown in Figure 1, while having no effect on the resting tension.

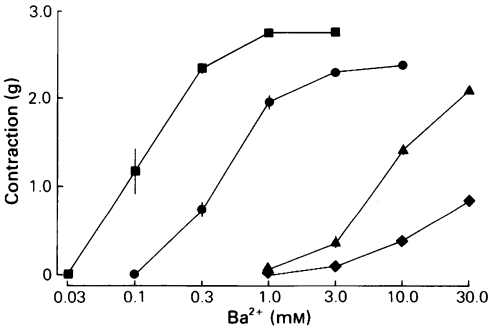

Figure 1 Effects of verapamil 10^{-6}M (▲), $LaCl_3$ 2.0 mM (◆) and Bay K8644 10^{-7} M (■) on the contraction induced by cumulative applications of $BaCl_2$ in rabbit aorta; (●) control. Agents to be tested were added 10 min before the addition of Ba^{2+}. Each curve was obtained on different muscle strips as described in the Methods. Each point represents mean of 4 experiments and s.e.mean is indicated by an error bar when it is greater than the symbol.

Ba^{2+}-induced contraction in Ca^{2+}-free solution

In a Ca^{2+}-free solution (containing 1.0 mM EGTA), cumulative application of 0.3–30.0 mM Ba^{2+} induced a concentration-dependent contraction (Figure 2). The concentration-response curves for Ba^{2+} in a Ca^{2+}-free solution were shifted to the right of the curve in a normal medium in a parallel manner without affecting the maximum tension. The second application of Ba^{2+} in a Ca^{2+}-free solution induced a contraction similar to the first Ba^{2+}-induced contraction (Figure 2).

Effects of verapamil (10^{-6} M), $LaCl_3$ (2.0 mM) and Bay K 8644 (10^{-7} M) on the Ba^{2+}-induced contraction in a Ca^{2+}-free solution are shown in Figure 3. The effects of these agents on the Ba^{2+}-induced contraction in the absence of external Ca^{2+} were similar to those in the presence of external Ca^{2+}; both verapamil and La^{3+} inhibited whereas Bay K 8644 augmented the Ba^{2+}-induced contraction. Nifedipine, 10^{-7} M, showed a similar inhibitory effect to verapamil 10^{-6} M (data not shown).

Effects of high K^+ and noradrenaline on the Ba^{2+}-induced contraction

Effects of high K^+ (65.4 mM) and noradrenaline (10^{-6} M) on the Ba^{2+}-induced contraction in a Ca^{2+}-free solution are shown in Figure 4. In a Ca^{2+}-free solution with high K^+, the concentration-response curve for Ba^{2+} shifted to the left. In a Ca^{2+}-free solution containing 10^{-6} M noradrenaline, however, the concentration-response curve for Ba^{2+} did not differ from the control curve obtained in a Ca^{2+}-free solution.

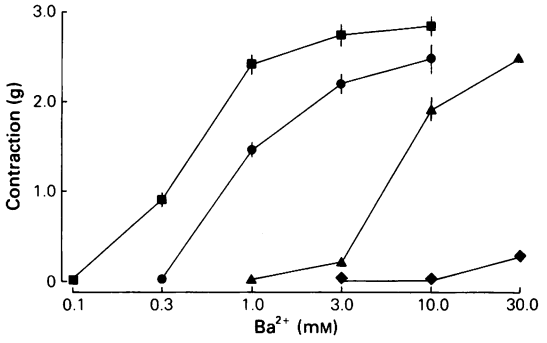

Figure 3 Effects of verapamil 10^{-6} M (▲), $LaCl_3$ 2.0 mM (◆) and Bay K8644 10^{-7} M (■) on the Ba^{2+}-induced contraction of rabbit aorta in a Ca^{2+}-free solution : (●) control. The muscle strip was incubated in a Ca^{2+}-free solution for 30 min and then Ba^{2+} was cumulatively applied. Agents to be tested were applied 10 min before the addition of Ba^{2+}. Each curve was obtained with different muscle strips as described in the Methods. Each point represents mean of 4 experiments; vertical lines show s.e.mean.

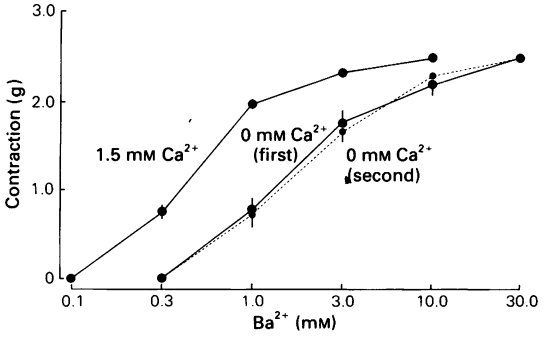

Figure 2 Effects of Ca^{2+}-free solution on the Ba^{2+}-induced contraction in the aorta. The muscle strip was incubated with a Ca^{2+}-free solution containing EGTA 1 mM for 30 min and then Ba^{2+} was applied cumulatively (first). After the application of Ba^{2+}, the muscle was washed with a high K^+, Ca^{2+}-free solution to obtain complete relaxation (see Methods). The muscle was then incubated with a Ca^{2+}-free solution for 10 min and then the second application of Ba^{2+} was performed (second). The control curve (1.5 mM Ca^{2+}) was obtained with different muscle strips as described in the Methods. Each point represents mean ± s.e.mean of 4 experiments.

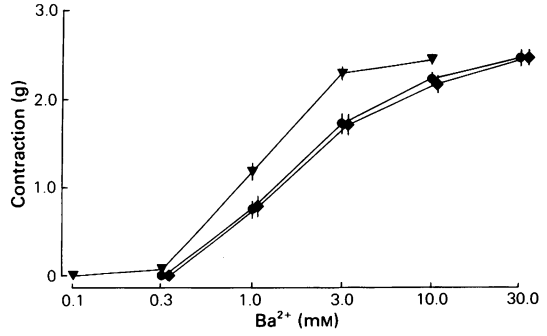

Figure 4 Effects of high concentration (65.4 mM) of K^+ and noradrenaline 10^{-6} M on the Ba^{2+}-induced contraction of rabbit aorta in a Ca^{2+}-free solution. The muscle strip was incubated with either a high K^+, Ca^{2+}-free solution (▼) or a Ca^{2+}-free solution containing noradrenaline for 30 min (◆) and then Ba^{2+} was cumulatively applied; (●) control. Each curve was obtained on different muscle strips as described in the Methods. Each point represents mean of 4 experiments; vertical lines show s.e.mean.

Effects of depletion of stored Ca^{2+} on the Ba^{2+}-induced contraction

In a Ca^{2+}-free solution, addition of noradrenaline (10^{-6} M) induced a transient contraction. The second application of noradrenaline induced only a small contraction, as shown in Figure 5, possibly because the Ca^{2+} store is depleted (Karaki *et al.*, 1979). In such a Ca^{2+}-depleted muscle, cumulative application of Ba^{2+} induced a contraction similar to that induced in a muscle without noradrenaline pretreatment. The second application of Ba^{2+} also induced a similar contraction to the first Ba^{2+}-induced contraction (Figure 5). Similar results were obtained when either caffeine (20.0 mM) or the simultaneous application of noradrenaline (10^{-6} M) and caffeine (20.0 mM) was used instead of noradrenaline (10^{-6} M) to deplete the cellular Ca^{2+} store (data not shown). The Ba^{2+}-induced contraction in the Ca^{2+}-depleted aorta was potentiated by Bay K 8644 and was inhibited by verapamil and La^{3+}, as was the case with the aorta simply treated with a Ca^{2+}-free solution in Figure 3.

Discussion

In the vascular smooth muscle of rabbit aorta, Ca^{2+} channel inhibitors like verapamil and nifedipine are known to inhibit selectively the voltage-dependent Ca^{2+} channel (Karaki & Weiss, 1984). In contrast, Bay K 8644, a Ca^{2+} channel facilitator (Schramm *et al.*, 1983), is reported to activate selectively the voltage-dependent Ca^{2+} channel (Yamamoto *et al.*, 1984). Further, La^{3+} is a nonselective inhibitor of Ca^{2+} influx in smooth muscle (Weiss, 1974). The finding that the Ba^{2+}-induced contraction in rabbit aorta was inhibited by verapamil, nifedipine and La^{3+} and was enhanced by Bay K8644 suggest that Ba^{2+} activates the voltage-dependent Ca^{2+} channels and increases Ca^{2+} influx to induce muscle contractions. In the intestinal smooth muscle of guinea-pig taenia coli, Ba^{2+} has been shown to increase both muscle tension and ^{45}Ca uptake (Karaki *et al.*, 1967, 1969). On the effect of Bay K 8644, this facilitator not only shifted the concentration-response curve for Ba^{2+} to the left but also increased the maximum contractile tension in the presence and absence of external Ca^{2+} (Figures 1 and 3). In contrast, high K^+-depolarization which also activates the voltage-dependent Ca^{2+} channel shifted the concentration-response curve for Ba^{2+} to the left but did not affect the maximum contractile tension (Figure 4). We do not have available data to explain this discrepancy except to suggest that Bay K 8644 might have other effects in addition to facilitation of voltage-dependant Ca^{2+} channels.

Influx of external Ca^{2+} is, however, only partly responsible for the Ba^{2+}-induced contraction in rabbit aorta because removal of external Ca^{2+} inhibited only a part of the Ba^{2+}-induced contraction. Present results indicate that, in a Ca^{2+}-free solution, Ba^{2+} enters the cell through the voltage-dependent Ca^{2+} channels; when the voltage-dependent Ca^{2+} channel is inhibited by verapamil, nifedipine or La^{3+}, the Ba^{2+}-induced contraction is inhibited and when the channel is

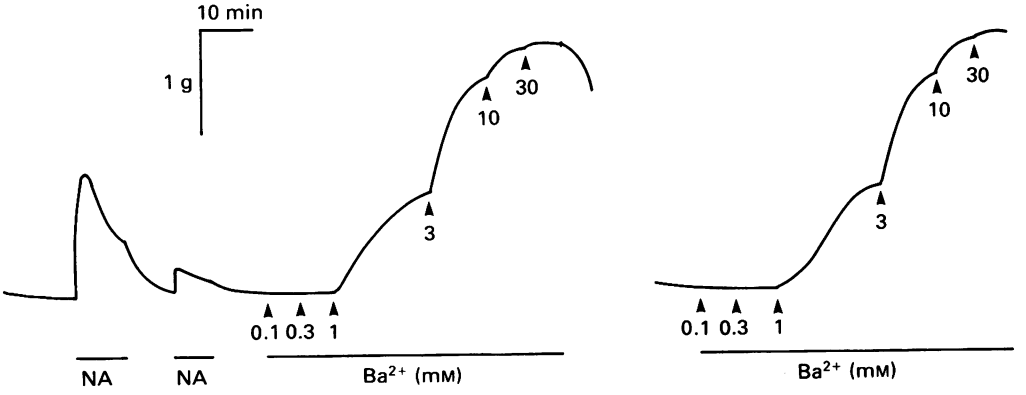

Figure 5 Effects of pretreatment with noradrenaline on the Ba^{2+}-induced contraction of rabbit aorta in a Ca^{2+}-free solution. The muscle was incubated in a Ca^{2+}-free solution for 10 min and then noradrenaline 10^{-6} M (NA) was applied; 10 min later, the muscle was washed with a Ca^{2+}-free solution for 10 min and then noradrenaline was applied again for another 10 min. The muscle was then washed with a Ca^{2+}-free solution and Ba^{2+} was cumulatively applied. After the completion of the cumulative application of Ba^{2+}, the muscle was washed with a high K^+, Ca^{2+}-free solution until complete relaxation was obtained (See Methods). The external medium was then changed to a Ca^{2+}-free solution and 10 min later, Ba^{2+} was applied again.

activated by Bay K 8644, the Ba^{2+}-induced contraction was enhanced. Further, the findings that high K^+ (which activates voltage-dependent Ca^{2+} channels), but not noradrenaline (which activates receptor-linked Ca^{2+} channels), enhanced the Ba^{2+}-induced contraction indicate that the receptor-linked Ca^{2+} channels are not permeable to Ba^{2+}. In various excitable cells including smooth muscle, it has been known that the voltage-dependent Ca^{2+} channels are more permeable to Ba^{2+} than to Ca^{2+} (Hagiwara, 1983; Yoshino & Yabu, 1985). Thus, the influx of Ba^{2+} through the voltage-dependent Ca^{2+} channel seems to be essential for the Ba^{2+}-induced contraction in the absence of external Ca^{2+}.

As a mechanism for the Ba^{2+}-induced contraction in the absence of external Ca^{2+}, one could speculate that intracellular Ba^{2+} mobilizes the cellular Ca^{2+} store. There is a cellular Ca^{2+} store in rabbit aorta which is mobilized by noradrenaline to induce a contraction. The amount of Ca^{2+} in the store is so small that application of noradrenaline induces only a transient contraction and since the store is depleted by a brief exposure to noradrenaline, the second application of noradrenaline is almost ineffective (Deth & van Breemen, 1977; Karaki *et al.*, 1979). Caffeine also depletes this Ca^{2+} store (Deth & Lynch, 1981; Leijten & van Breemen, 1984). In vascular smooth muscle, Bond *et al.* (1984) reported that sarcoplasmic reticulum is the only intracellular Ca^{2+} store and both noradrenaline and caffeine release Ca^{2+} from this store. In the present experiments, it was found that, in the aorta of which the Ca^{2+} store had previously been depleted by the repeated applications of noradrenaline and/or caffeine, Ba^{2+} still induced a sustained contraction similar to that in the muscle without such pretreatment. Further, the first and the second applications of Ba^{2+} induced similar contractions in the Ca^{2+}-depleted muscle as well as in the non-depleted muscle. These results suggest that the Ca^{2+} store which is mobilized by noradrenaline or caffeine is not involved in the Ba^{2+}-induced contraction. However, these results do not exclude the possibility that Ba^{2+} mobilizes the Ca^{2+} store which is not depleted by noradrenaline and caffeine.

An alternative possibility is that Ba^{2+} entering the cell might directly activate the contractile machinery. In fact, Ba^{2+} is able to induce super-precipitation of smooth muscle actomyosin (Ebashi & Kodama, 1967) and also to contract the glycerin-treated smooth muscle of guinea-pig taenia coli (Bando *et al.*, 1970); the ability of Ba^{2+} to activate the contractile protein in smooth muscle is 1/79 (in the glycerin-treated guinea-pig taenia) or 1/290 (in the super-precipitation of chicken gizzard actomyosin) that of Ca^{2+}. In vascular smooth muscle, 10^{-6} M intracellular Ca^{2+} induces full activation of the contractile protein (Filo *et al.*, 1965). Therefore, maximum contraction could be elicited if the smooth muscle cells could accumulate 8×10^{-5} M to 3×10^{-4} M Ba^{2+} ($10^{-6} \times 79-290$). In rabbit aorta, 65.4 mM K^+ induces nearly maximum contraction in the presence of 0.5 mM Ca^{2+} (unpublished observation) when the intracellular Ca^{2+} would be approximately 10^{-6} M and the transmembrane concentration gradient of Ca^{2+} may be approximately 1/500. In a Ca^{2+}-free solution, 30 mM Ba^{2+} induces a nearly maximum contraction and if the transmembrane concentration gradient of Ba^{2+} were the same as Ca^{2+}, the intracellular Ba^{2+} would be 6×10^{-5} M ($3 \times 10^{-2} \times 1/500$). Taking account of the fact that the voltage-dependent Ca^{2+} channel is more permeable to Ba^{2+} than to Ca^{2+} (Hagiwara *et al.*, 1983), it seems possible that the concentration of intracellular Ba^{2+} increases to the level directly activating the contractile machinery, although further studies are needed to obtain direct evidence.

It is concluded that Ba^{2+} seems to open the voltage-dependent Ca^{2+} channels and Ca^{2+} may enter the cell to induce contraction in the vascular smooth muscle of rabbit aorta. In the absence of Ca^{2+}, however, Ba^{2+} itself may enter the cell and induce contraction without mobilizing the Ca^{2+} store which is sensitive to noradrenaline and caffeine.

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References

BANDO, T. AIZU, M., SAKATO, U. & YANAGISAWA, M. (1970). Pharmacology of divalent ions on smooth muscle. *Folia pharmac. jpn.*, **66**, 89p. (in Japanese)

BOND, M., KITAZAWA, T., SOMLYO, A.P. & SOMLYO, A.V. (1984). Release and recycling of calcium by the sarcoplasmic reticulum in guinea-pig portal vein smooth muscle. *J. Physiol.*, **355**, 677–695.

BÜLBRING, E. & TOMITA, T. (1969). Effects of calcium, barium and manganese on the action of adrenaline in the smooth muscle of guinea-pig taenia coli. *Proc. R. Soc. B.*, **172**, 121–136.

DANIEL, E.E. (1963). On the role of calcium, strontium and barium in contraction and excitability of rat uterine muscle. *Archs. int. Pharmacodyn. Thér.*, **146**, 298–349.

DETH, R.C. & LYNCH, C. (1981). Mobilization of a common source of smooth muscle Ca^{2+} by norepinephrine and methylxanthies. *Am. J. Physiol.*, **240**, C239–C247.

DETH, R. & VAN BREEMEN, C. (1977). Agonist induced release of intracellular Ca^{2+} in the rabbit aorta. *J. membrane Biol.*, **30**, 363–380.

EBASHI, S. & KODAMA, F. (1967). Effects of alkaline earth

metal ions on the contractile system with special reference to troponin. *Folia pharmac. ipn.*, **63,** 172p (in Japanese)

FILO, R.S., BOHR, D.F. & RUEGG, J.C. (1965). Glycerinated skeletal and smooth muscle: calcium and magnesium dependence. *Science*, **147,** 1581–1583.

HAGIWARA, S. (1983). *Membrane Potential-Dependent Ion Channels in Cell Membrane.* New York: Raven Press.

HOTTA, Y. & TSUKUI, K. (1968). Effect on the guinea-pig taenia coli of the substitution of strontium and barium ions for calcium ions. *Nature*, **217,** 867–869.

KARAKI, H., IKEDA, M. & URAKAWA, N. (1967). Effects of external calcium and some metabolic inhibitors on barium-induced tension changes in guinea pig taenia coli. *Jap. J. Pharmac.*, **17,** 603–612.

KARAKI, H., IKEDA, M. & URAKAWA, N. (1969). Movements of calcium during tension development induced by barium and high-potassium in guinea pig taenia coli. *Jap. J. Pharmac.*, **19,** 291–299.

KARAKI, H., KUBOTA, H. & URAKAWA, N. (1979). Mobilization of stored calcium for phasic contraction induced by norepinephrine in rabbit aorta. *Eur. J. Pharmac.*, **56,** 237–245.

KARAKI, H., NAKAGAWA, H. & URAKAWA, N. (1984). Effects of calcium antagonists on release of [^{3}H]noradrenaline in rabbit aorta. *Eur. J. Pharmac.*, **101,** 177–183.

KARAKI, H., SHIBATA, S. & SATAKE, N. (1986). Mechanism of barium-induced contraction in vascular smooth muscle. *Fedn. Proc.*, **45,** 671.

KARAKI, H., SUZUKI, T. & URAKAWA, N. (1981). Tris does not inhibit isolated vascular or intestinal smooth muscle contraction. *Am. J. Physiol.*, **241,** H337–H341.

KARAKI, H. & URAKAWA, N. (1977). Possible role of endogenous catecholamines in the contractions induced in rabbit aorta by ouabain, sodium depletion and potassium depletion. *Eur. J. Pharmac.*, **43,** 65–72.

KARAKI, H. & WEISS, G.B. (1984). Calcium channels in smooth muscle. *Gastroenterology*, **87,** 960–970.

LEIJTEN, A.A. & VAN BREEMEN, C. (1984). The effects of caffeine on the noradrenaline-sensitive calcium store in rabbit aorta. *J. Physiol.*, **357,** 327–339.

SCHRAMM, M., THOMAS, G., TOWART, R. & FRANCKOWIAK, G. (1983). Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature*, **303,** 535–537.

SOMLYO, A.P., SOMLYO, A.V., DEVINE, C.E., PETERS, P.D. & HALL, T.A. (1974). Electron microscopy and electron probe analysis of mitochondrial cation accumulation in smooth muscle. *J. cell Biol.*, **61,** 723–742.

SUZUKI, T., NISHIYAMA, A. & OKAMURA, K. (1964). The effects of barium ion on the resting and action potential of intestinal smooth muscle cells. *Tohoku J. exp. Med.*, **82,** 87–92.

WEISS, G.B. (1974). Cellular pharmacology of lanthanum. *A. Rev. Pharmac.*, **14,** 343–354.

YAMAMOTO, H., HWANG, O. & VAN BREEMEN, C. (1984). Bay K8644 differentiates between potential and receptor operated Ca^{2+} channels. *Eur. J. Pharmac.*, **102,** 555–557.

YOSHINO, M. & YABU, H. (1985). Single Ca channel currents in mammalian visceral smooth muscle cells. *Pflügers Arch.*, **404,** 285–286.

YUKISADA, N. & EBASHI, F. (1961). Role of calcium in drug action on smooth muscle. *Jap. J. Pharmac.*, **11,** 46–53.

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