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Ca^{2+} entry activated by emptying of intracellular Ca^{2+} stores in ileal smooth muscle of the rat

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1 The effects of depletion of intracellular Ca^{2+} stores on muscle tension and the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were studied in fura-2 loaded longitudinal smooth muscle cells of the rat ileum.

2 After exposure to a Ca^{2+}-free solution, application of Ca^{2+} caused a small contraction and a rise in $[Ca^{2+}]_i$, both of which were potentiated when the muscle was challenged with carbachol or caffeine before the addition of Ca^{2+}.

3 Cyclopiazonic acid (CPA), a specific inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, dose-dependently decreased tension development and the rises in $[Ca^{2+}]_i$ induced by carbachol and caffeine in the Ca^{2+}-free solution, but conversely increased the Ca^{2+}-induced responses even in the presence of the voltage-dependent Ca^{2+} channel blockers, methoxyverapamil and nifedipine.

4 The contraction and rise in $[Ca^{2+}]_i$ evoked by Ca^{2+} gradually declined with time after removal of CPA, while the reverse was the case for the responses to carbachol and caffeine.

5 The Ca^{2+}-induced contraction and rise in $[Ca^{2+}]_i$ in the presence of CPA were inhibited by the replacement of Na^+ with K^+ or Cs^+, and by the addition of Cd^{2+}, Ba^{2+}, Ni^{2+} or La^{3+}.

6 The influx of Mn^{2+} was much greater in extent in the presence of CPA than in its absence.

7 These results suggest that the emptying of intracellular Ca^{2+} stores may activate Ca^{2+} influx not associated with voltage-dependent Ca^{2+} channels in the rat ileal smooth muscle.

Keywords: Ca^{2+} influx; cyclopiazonic acid; intestinal smooth muscle; intracellular Ca^{2+} stores; Mn^{2+}-quenching

Introduction

A rise in the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) is essential for evoking contractile responses in smooth muscle. This activator Ca^{2+} is either released from intracellular Ca^{2+} stores that possess Ca^{2+}-release channels activated by inositol 1,4,5-trisphosphate (IP_3) (Somlyo *et al.*, 1985; Hashimoto *et al.*, 1986) and Ca^{2+} itself (Iino, 1989), or enters into the cells through voltage-dependent Ca^{2+} channels and receptor-operated ones (Bolton, 1979). Recently, the 'capacitative Ca^{2+} entry' hypothesis, postulating that a decrease in the Ca^{2+} content of the intracellular Ca^{2+} stores is sufficient to trigger the Ca^{2+} influx in non-excitable cells, has been proposed (Putney, 1986; 1990). A similar phenomenon has also been observed in vascular (Missiaen *et al.*, 1990; Xuan *et al.*, 1992; Noguera & D'Ocon, 1993; Pacaud *et al.*, 1993) and urinary smooth muscle (Munro & Wendt, 1994). In the rat intestinal smooth muscle, we found that Ca^{2+} caused a large increase in muscle tension, when added after depletion of intracellular Ca^{2+} stores by Ca^{2+}-releasing agents under Ca^{2+}-free conditions. Therefore, it is possible that the 'capacitive Ca^{2+} entry' mechanism is present in this tissue. Nevertheless, in visceral smooth muscle, there is little information about the presence of such a regulatory mechanism for intracellular Ca^{2+} stores.

Cyclopiazonic acid (CPA) has been shown to inhibit the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase pump selectively (Seidler *et al.*, 1989), thereby preventing the uptake of Ca^{2+} into SR. It has also been reported in smooth muscle that CPA promotes depletion of Ca^{2+} from the stores by functionally inhibiting refilling (Bourreau *et al.*, 1991; Shima & Blaustein, 1992; Uyama *et al.*, 1992; Kasai *et al.*, 1994; Munro & Wendt, 1994). CPA, therefore, has been used as a pharmacological tool to study the functional role of Ca^{2+} stores (Darby *et al.*, 1993).

The aim of the present experiments is to determine whether Ca^{2+} stores regulate Ca^{2+} entry, dependent on their filling state in intestinal smooth muscle of the rat. For this purpose, we measured isometric tension and $[Ca^{2+}]_i$ simultaneously in fura-2 loaded tissues. The effects of depletion of store Ca^{2+} induced by CPA on Ca^{2+}-induced contractile responses and $[Ca^{2+}]_i$ responses were also examined. We have already reported that this muscle has both IP_3- and caffeine sensitive Ca^{2+} stores that are functionally important in mediating contractile and membrane current responses (Ito *et al.*, 1993; Ohta *et al*, 1993; 1994). In the present study, we have demonstrated that the emptying of the Ca^{2+} stores induced by CPA initiated a considerable rise in $[Ca^{2+}]_i$ and the contractile response, dependent on the presence of extracellular Ca^{2+}.

Methods

Male Wistar rats (200–300 g) were stunned and bled to death. The ileum was excised and luminal contents were removed by washing with normal physiological salt solution (PSS). The longitudinal muscle layer was peeled from the underlying circular muscle layer and thin muscle strips (1 mm in width, 8 mm in length) were dissected. Then they were incubated with 20 μM fura-2 acetoxymethyl ester (fura-2/AM) and 0.02% cremophore EL, a noncytotoxic detergent, for more than 3 h at room temperature for simultaneous measurement of the contractile activity and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Experiments were carried out with a fluorimeter (CAF-110, Japan Spectroscopic) at room temperature (22–25°C). The muscle strip was held horizontal to the experimental chamber (volume about 0.2 ml) : one end of the muscle strip was fixed with pins to the silicon rubber at the edge of the bottom of the chamber, and the other end was connected to a strain gauge transducer to measure the isometric tension. The bathing solutions were changed by rapidly injecting 5 ml of solution and removing the overflow

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by suction. The muscle strips were alternately illuminated by 340 nm and 380 nm light (128 Hz) and the intensity of fluorescence at 500 nm was measured. The ratio of the fluorescence due to excitation at 340 nm to that at 380 nm (F340/F380) was calculated from successive illumination periods and was considered to be an index of $[Ca^{2+}]_i$.

Normal PSS contained (mM): NaCl 144, KCl 5.8, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.1, HEPES 5 (pH 7.4 with NaOH). Ca^{2+}-free solution was made by omitting $CaCl_2$ and adding 2 mM EGTA. To determine the effects of Na^+ removal, NaCl was isosmotically replaced by KCl, LiCl or CsCl.

The following chemicals were used: caffeine and methoxyverapamil (Wako Pure. Chem.), carbachol, cremophore EL and cyclopiazonic acid (Sigma), EGTA, Fura-2/AM and HEPES (Dojindo), and nifedipine (Bayer).

Results of the experiments are expressed as the mean ± s.e.mean. Student's t test was used for statistical analysis of the results and $P<0.05$ was considered to indicate a significant difference.

Results

Effects of Ca^{2+}-store release on contraction and $[Ca^{2+}]_i$ induced by application of Ca^{2+}

In the rat ileal longitudinal smooth muscle loaded with fura-2, the contraction and rise in $[Ca^{2+}]_i$ induced by application of Ca^{2+} were observed before and after stimulation with carbachol or caffeine in the Ca^{2+}-free solution. Representative results are shown in Figure 1. After tissues had been incubated with Ca^{2+}-free solution for 7 min, the reintroduction of Ca^{2+} (2.5 mM) produced a small contraction associated with a rise in $[Ca^{2+}]_i$. Two minutes after removal of the external Ca^{2+}, the administration of carbachol (0.1 mM) or caffeine (30 mM) to Ca^{2+}-free solution resulted in a transient contraction and a rise in $[Ca^{2+}]_i$ mediated by Ca^{2+} released from the intracellular Ca^{2+} stores. After washout with fresh Ca^{2+}-free solution, no response was evoked by either carbachol or caffeine applied subsequently, indicating that the intracellular Ca^{2+} stores had been depleted. In such tissues with depleted Ca^{2+} stores, both contractile and $[Ca^{2+}]_i$ responses to the application of Ca^{2+} were markedly enhanced. The contraction and rise in $[Ca^{2+}]_i$ induced by Ca^{2+}-application were enhanced 2.6 ± 0.8 and 1.4 ± 0.3 fold (n = 8), respectively, by the preceding stimulation with caffeine, and 2.2 ± 0.4 and 1.6 ± 0.3 fold (n = 8) with carbachol.

Effects of cyclopiazonic acid on the content of stored Ca^{2+} and on the response to Ca^{2+}-application

To determine the relationship between the filling state of the Ca^{2+} stores and the magnitude of Ca^{2+}-induced responses, the effect of cyclopiazonic acid (CPA) on the responses to Ca^{2+}-application and subsequent carbachol or caffeine was observed. After the control responses were obtained, tissues were treated with various concentrations of CPA for 26 min. At 20 min after the start of CPA treatment, the tissues were exposed to 2.5 mM Ca^{2+} for 3 min to load Ca^{2+} into intracellular stores and were then washed for 2 min with Ca^{2+}-free solution containing 2 mM EGTA. Subsequently, carbachol or caffeine was applied for 1 min under Ca^{2+}-free conditions. The Ca^{2+} content in the stores was estimated by measuring the amplitude of the response to carbachol or caffeine. As shown in Figure 2, 0.3 μM CPA had almost no effect on the contractile and $[Ca^{2+}]_i$ responses to either carbachol or Ca^{2+}-application. CPA over 1 μM caused a decrease in the responses to carbachol and an increase in responses to Ca^{2+} in a concentration-dependent manner. These effects of CPA gradually recovered after washout of the drug as mentioned below. A typical experimental result on the effect of CPA (10 μM) is depicted in Figure 3. In the presence of CPA, the responses to Ca^{2+} were sustained during its application and quickly returned to the original level after its removal. The relationships between the responses to Ca^{2+} and those to carbachol were inverse; that is, the smaller the responses to carbachol, the larger the responses to Ca^{2+}-application. Qualitatively the same result was obtained when caffeine was used instead of carbachol. All these experiments were carried out in the presence of methoxyverapamil (10 μM) or nifedipine (1 μM) to eliminate the possible involvement of voltage-dependent Ca^{2+} channels.

When CPA was withdrawn from the bathing solution, the contraction and rise in $[Ca^{2+}]_i$ evoked by the application of Ca^{2+} gradually declined, dependent on the time after removal of CPA, while the reverse was the case for the responses to

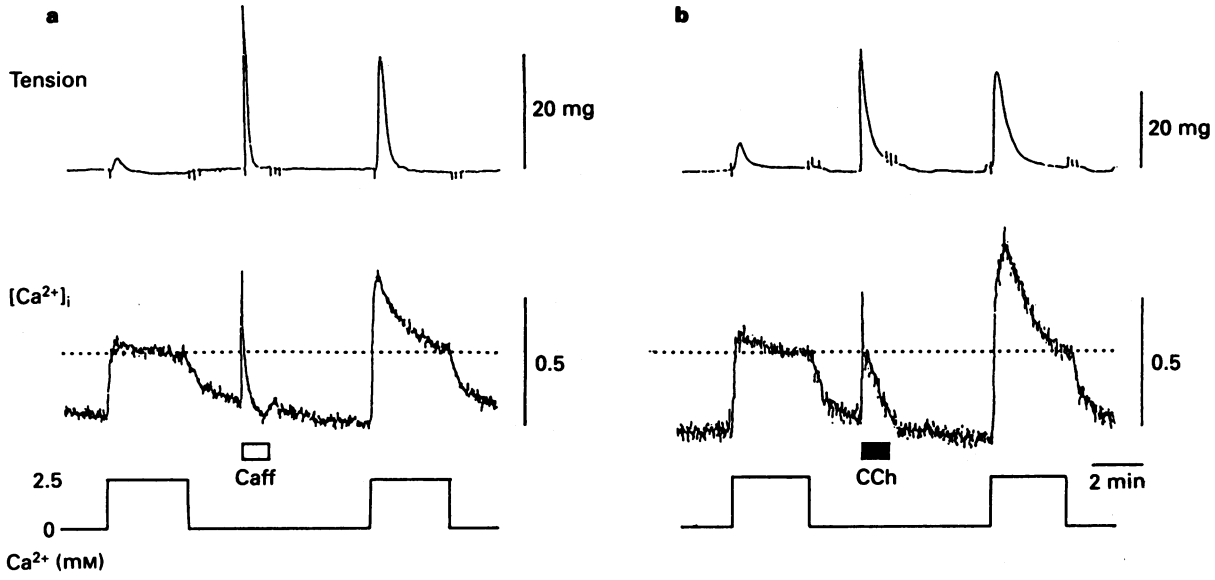

Figure 1 Effects of the preceding application of caffeine (a) and carbachol (b) on the contraction and rise in $[Ca^{2+}]_i$ induced by application of Ca^{2+}. Traces from top to bottom: tension, $[Ca^{2+}]_i$ and the concentration of external Ca^{2+}. The tissues were incubated with Ca^{2+}-free solution for 7 min before the first reintroduction of Ca^{2+} (2.5 mM) for 3 min. Then the tissues were washed for 2 min with Ca^{2+}-free solution containing 2 mM EGTA and were stimulated by caffeine (Caff, □ ; 30 mM) or carbachol (CCh, ■ ; 0.1 mM) for 1 min from 5 min before the second application of Ca^{2+}. Dotted lines indicate the resting $[Ca^{2+}]_i$ level in the normal PSS.

carbachol (Figure 3). The same results were obtained in 3 other experiments and when caffeine was used instead of carbachol. These results indicate that CPA promotes depletion of Ca^{2+} from the stores and potentiates Ca^{2+}-induced responses, suggesting that the Ca^{2+} content in the stores may regulate Ca^{2+} entry into rat intestinal smooth muscle cells.

Effects of CPA on Mn^{2+} influx

It has been shown that Mn^{2+} is a good substitute for Ca^{2+} in defining Ca^{2+} entry pathways, because it can pass through almost all the Ca^{2+} permeable channels but cannot be a substrate for the sarcoplasmic reticulum pump (Gomes De Costa & Madeira, 1986; Missiaen *et al.*, 1990). Therefore, to obtain more direct evidence that Ca^{2+} depletion in the stores promotes Ca^{2+} entry into the cell, Mn^{2+} influx was monitored by Mn^{2+} quenching of fura-2 fluorescence. These experiments were carried out in the presence of methoxyverapamil (10 μM). After tissues were incubated with nominal Ca^{2+}-free solution with or without CPA (10 μM) for 20 min, the external solution was replaced with solution containing Mn^{2+} (0.1 mM). The time courses of Mn^{2+}-induced fura-2 quenching in the presence and absence of CPA are shown in Figure 4. Although the quenching of fura-2 was initiated even in the absence of CPA, the rate of quenching was much greater in the presence of CPA than in its absence. These results suggest that Ca^{2+} permeability of the plasma membrane may be increased by CPA.

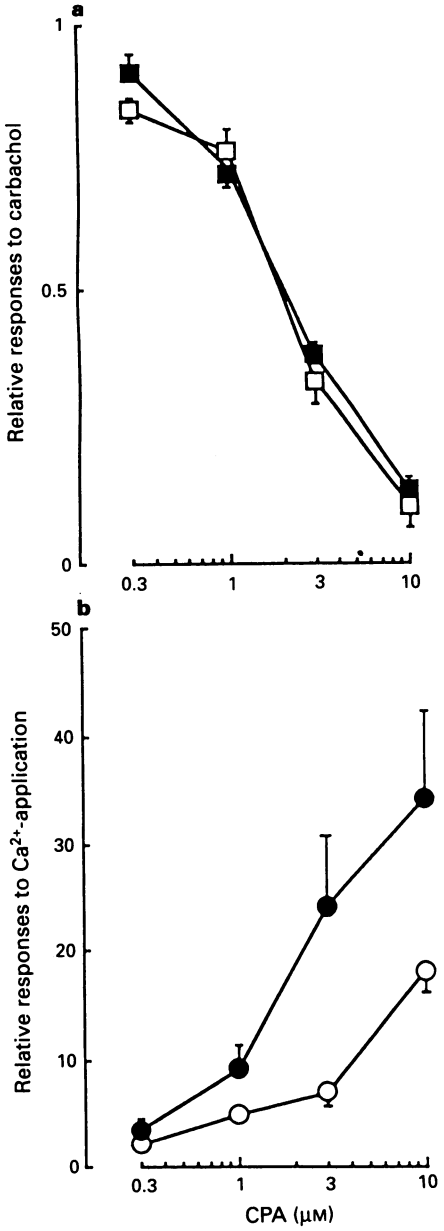

Figure 2 The cyclopiazonic acid (CPA)-evoked concentration-dependent decreases in the responses to carbachol (0.1 mM, a) and increases in responses to Ca^{2+} (2.5 mM, b). The tissues were incubated with each given concentration of CPA for 26 min. The amplitudes (a) and area (b) of the contractile (open symbols) and $[Ca^{2+}]_i$ (filled symbols) responses are plotted against a given concentration of CPA as a percentage of those obtained in the absence of CPA (mean ± s.e. mean, $n = 4$). In the case of the rise of $[Ca^{2+}]_i$ induced by Ca^{2+}, the area above the resting level in normal PSS (2.5 mM Ca^{2+}) was measured. Methoxyverapamil (10 μM) was present throughout the experiments.

Effects of metal ions and replacement of Na^+ with other monovalent cations on $[Ca^{2+}]_i$ and contractile responses in the presence of CPA

Tissues were pretreated with CPA (10 μM) for 20 min and experiments were carried out in its presence. After a sustained rise in $[Ca^{2+}]_i$ and contraction had been evoked by Ca^{2+} (2.5 mM), metal ions were cumulatively added to the bathing solution. Figure 5a is the original traces showing the concentration-dependent inhibitory action of La^{3+}. With this experimental protocol, the concentration-inhibition curves for Cd^{2+}, Ba^{2+}, Ni^{2+} and La^{3+} were constructed and are shown in Figure 5b. All these metal ions dose-dependently inhibited the rise in $[Ca^{2+}]_i$ and contraction induced by Ca^{2+}-application. La^{3+} was the most potent ion and was effective at less than 1 μM. The potency order was $La^{3+} > Ni^{2+} = Ba^{2+} > Cd^{2+}$.

Figure 5c shows the effects of isosmotic replacement of external Na^+ with K^+, Cs^+ or Li^+. Complete substitution of Li^+ for Na^+ slightly increased $[Ca^{2+}]_i$ and contractile responses to Ca^{2+}-application, but that with Cs^{2+} decreased both responses. Replacement of Na^+ with K^+ showed a much greater effect on these responses: increases of K^+ concentrations dose-dependently decreased the rise in $[Ca^{2+}]_i$ and contraction induced by Ca^{2+}-application.

Discussion

The present experiments showed that transient contractions and rises in $[Ca^{2+}]_i$ induced by carbachol and caffeine in Ca^{2+}-free solution were inhibited by a specific sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor, CPA (Seidler *et al.*, 1989) in the longitudinal smooth muscle of the rat ileum, suggesting that Ca^{2+} uptake into the stores is mediated by Ca^{2+}-ATPase pump protein as reported in other smooth muscles (Bourreau *et al.*, 1991; Shima & Blaustein, 1992; Uyama *et al.*, 1992; Kasai *et al.*, 1994; Muro & Wendt, 1994). On the other hand, CPA markedly augmented the rise in $[Ca^{2+}]_i$ and contraction in response to Ca^{2+}-application even in the presence of voltage-dependent Ca^{2+} channel blockers. Both in different concentrations of CPA and during recovery from CPA action there was an inverse correlation between the inhibitory effects on the responses mediated by Ca^{2+} released from the stores, and the potentiating effects on those induced by Ca^{2+}-application.

Since the potentiating effect of CPA on responses to Ca^{2+}-application was still observed in solutions containing Li^+ instead of external Na^+, it is unlikely that depression of the Na^+/Ca^{2+} exchange mechanism contributes to the potentiating effects of CPA. Replacement of Na^+ by Cs^+ or K^+ decreased the CPA-induced potentiation of responses and this inhibitory effect was greater when Na^+ was replaced by K^+. It is well-known that an increase in the external K^+ concentration initiates membrane depolarization (Bolton, 1979) and the same is true for Cs^+ (Sjodin, 1959). Therefore, the inhibitory effect of external K^+ on the responses induced

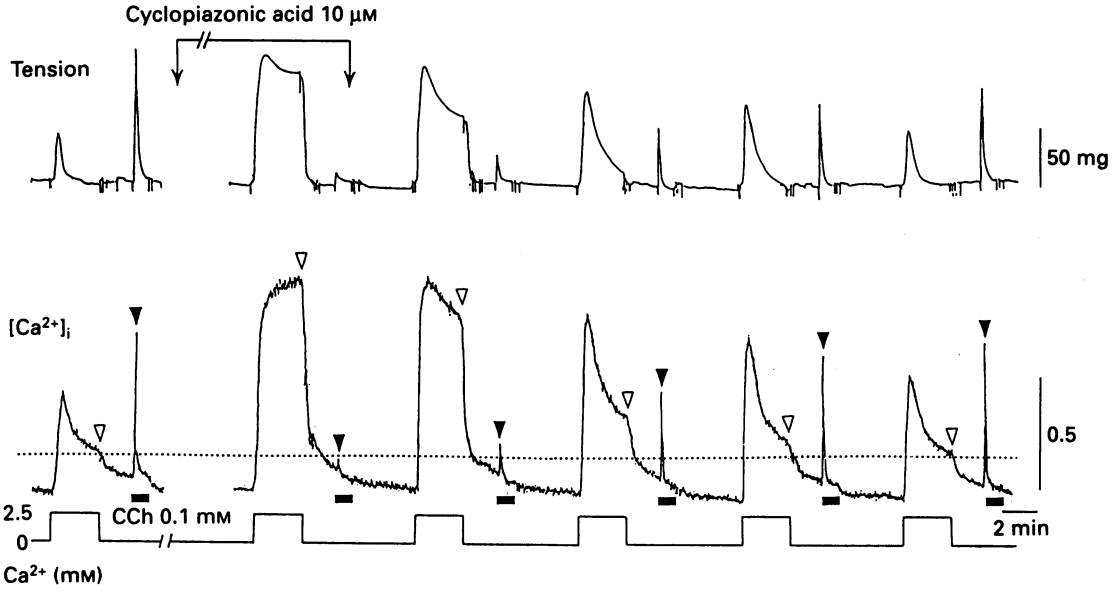

Figure 3 Representative responses to carbachol and Ca^{2+}-application before, during and after the addition of cyclopiazonic acid (CPA). Tissues were treated with CPA (10 μM) for 26 min during the period indicated at the top of the trace. At 20 min after the start of CPA treatment, the tissues were exposed to 2.5 mM Ca^{2+} for 3 min and were then washed for 2 min with Ca^{2+}-free solution containing 2 mM EGTA. Subsequently carbachol (0.1 mM, ■ , 1 min) was applied under Ca^{2+}-free condition. Methoxyverapamil (10 μM) was present throughout the experiments. The dotted line indicates the resting $[Ca^{2+}]_i$ level in normal PSS. ▽ : $[Ca^{2+}]_i$ at 3 min after the application of Ca^{2+}; ▼: peak $[Ca^{2+}]_i$ induced by carbachol in Ca^{2+}-free solution.

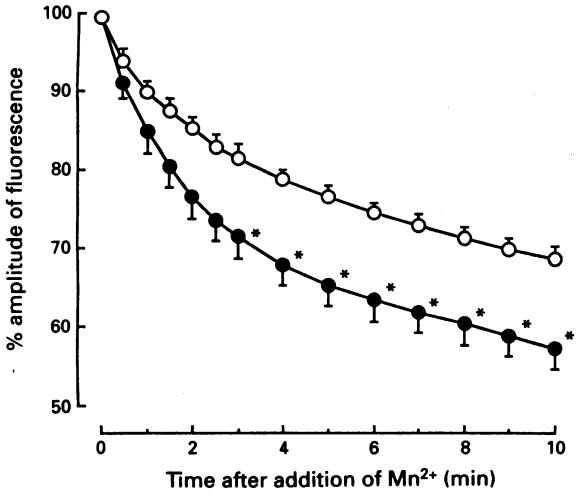

Figure 4 Effects of cyclopiazonic acid (CPA) on Mn^{2+} influx. The time course of quenching of fura-2 fluorescence due to Mn^{2+} influx was monitored in the presence (●, $n = 5$) and absence (○, n = 5) of CPA (10 μM). The amplitude of the fluorescent signal excited at 360 nm (isosbestic point of fura-2) after the addition of Mn^{2+} (0.1 mM) to the nominal Ca^{2+}-free solution is expressed as a percentage of that before the addition of Mn^{2+}. *Significantly different from the control value at $P<0.05$.

by depletion of the Ca^{2+} stores might be due to the reduction of the electrochemial gradient for Ca^{2+} across the cell membrane. A similar phenomenon has been reported in voltage-clamped smooth muscle cells (Pacaud & Bolton, 1991; Pacaud *et al.*, 1993). Furthermore, some metal ions that are known to affect Ca^{2+} flux through the plasma membrane blocked the CPA-induced rise in $[Ca^{2+}]_i$ and contraction in a concentration-dependent manner. These results suggest that the content of Ca^{2+} in the stores may regulate Ca^{2+} entry in rat intestinal smooth muscle.

Most reports concerning Ca^{2+} entry into vascular smooth muscle cells show that Mn^{2+} influx is potentiated by emptying of Ca^{2+} stores (Jacob, 1990; Missiaen *et al.*, 1990; Xuan *et al.*, 1992). Similarly, in the present experiments, acceleration of Mn^{2+} influx was observed in tissues treated with CPA. However, it has been reported that treatments leading to the depletion of Ca^{2+} stores fail to increase Mn^{2+} influx in the rabbit inferior vena cava, suggesting that the rise in $[Ca^{2+}]_i$ resulting from the depletion of the Ca^{2+} stores is due to a disturbance of the superficial buffer barrier system rather than to the activation of Ca^{2+} entry (Chen & van Breeman, 1993). Although we cannot exclude the existence of such a system in the rat ileal smooth muscle, the potentiating effects of store depletion on the responses to Ca^{2+}-application were observed even in tissues with functionally intact Ca^{2+} stores. Since the Ca^{2+} content in the stores must be much less in tissues challenged with Ca^{2+}-releasing agents, the buffering-action capacity of the stores in these tissues should be stronger than in those without Ca^{2+}-releasing stimulation. If so, the rise in $[Ca^{2+}]_i$ should have been small in the tissues stimulated by Ca^{2+}-releasing drugs. Since this was not the case, the contribution of Ca^{2+} buffering, if any, must be small in the rat ileal smooth muscle. The extent of its contribution may be dependent on the different preparations and tissues, because in some smooth muscles a procedure leading to the emptying of Ca^{2+} stores failed to increase $[Ca^{2+}]_i$ (Shima & Blaustein, 1992; Kasai *et al.*, 1994).

Although the precise mechanism of Ca^{2+} entry induced by the depletion of Ca^{2+} stores remains unsolved, several substances have recently been reported to be mediators for this mechanism such as the 'Ca^{2+}-influx factor' found in lymphocyte cell lines (Randriamampita & Tsien, 1993), a phosphatase-related diffusible messenger in *Xenopus* oocytes (Parekh *et al.*, 1993) and a small GTP binding protein in basophilic leukaemia cells (Fasolato *et al.*, 1993) and lacrimal acinar cells (Bird & Putney, 1993). Furthermore, a Ca^{2+}-selective current activated by depletion of intracellular Ca^{2+} stores (I_{CRAC}) has been recorded in mast cells (Hoth & Penner, 1992; Fasolato *et al.*, 1993). Therefore, further biochemical and electrophysiological analyses are necessary to identify the mechanism of Ca^{2+} entry activated by emptying the Ca^{2+} stores in rat intestinal smooth muscle cells.

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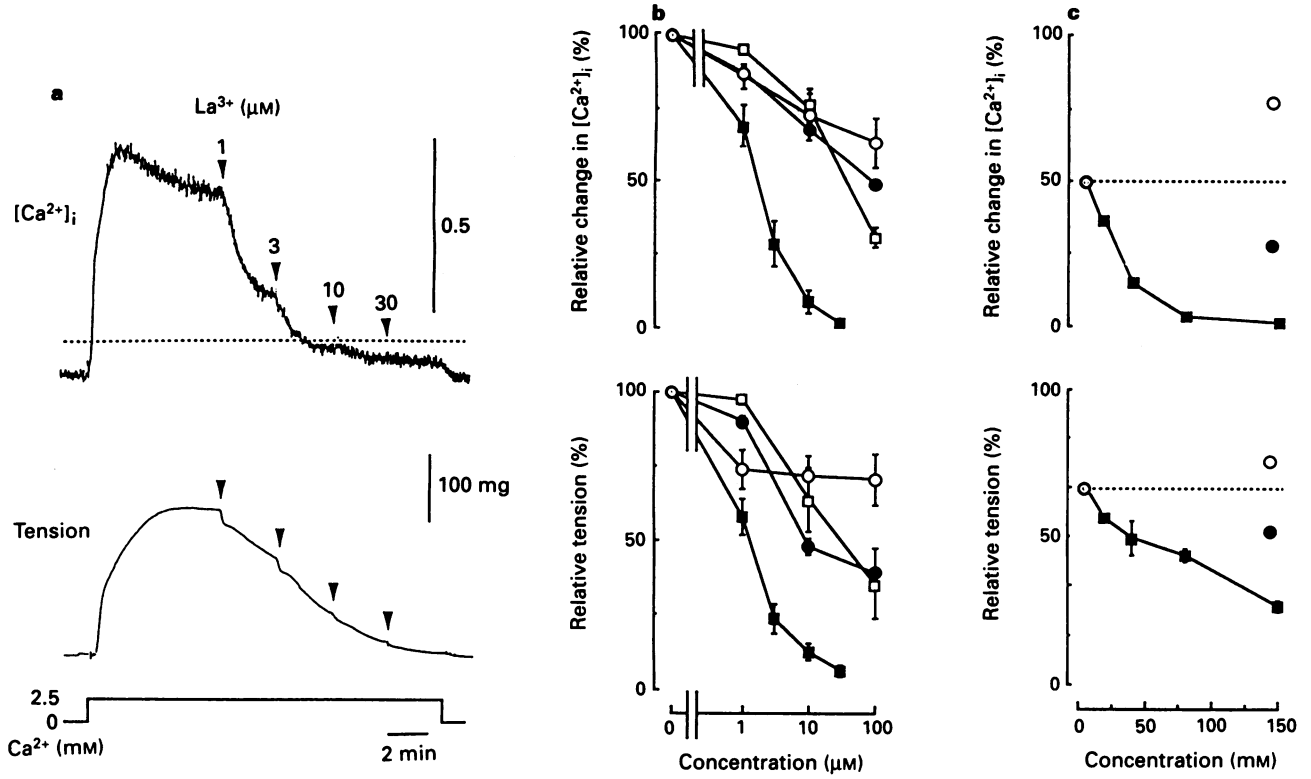

Figure 5 Effects of Cd^{2+}, Ba^{2+}, Ni^{2+} and La^{3+}, and of the replacement of Na^+ with Cs^+, K^+ and Li^+ on the contractile and $[Ca^{2+}]_i$ responses induced by application of Ca^{2+} (2.5 mM) in the presence of cyclopiazonic acid (CPA 10 μM). (a) The original traces showing concentration-dependent inhibitory action of La^{3+}. After the responses to Ca^{2+}-application attained a constant level, La^{3+} was added cumulatively. Traces from top to bottom: tension, $[Ca^{2+}]_i$ and the concentration of external Ca^{2+}. (b) Concentration-response relationships for metal ions: (○) Cd^{2+}; (●) Ba^{2+}; (□) Ni^{2+} and (■) La^{3+}. (c) The effects of Na^+ replacement with other monovalent cations on the contractile and $[Ca^{2+}]_i$ responses induced by application of Ca^{2+}: (○) Li^+; (●) Cs^+ and (■) K^+. Amplitude of the evoked flurorescent signal above the basal level (in the absence of Ca^{2+}) was measured. The amplitude of each response was plotted against the concentration of ions as a percentage of the control (mean ± s.e.mean, $n = 4$). Methoxyverapamil (10 μM) was present throughout the experiments.

References

BIRD, G.St.J. & PUTNEY, J.W. Jr. (1993). Inhibition of thapsigargin-induced calcium entry by microinjected guanine nucleotide analogues. *J. Biol. Chem.*, **268**, 21486–21488.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BOURREAU, J.P., ABELA, A.P., KWAN, C-Y. & DANIEL, E.E. (1991). Acetylcholine Ca^{2+} stores refilling directly involves a dihydropyridine-sensitive channel in dog trachea. *Am. J. Physiol.*, **261**, C497–C505.

CHEN, Q. & VAN BREEMEN, C. (1993). The superficial buffer barrier in venous smooth muscle: sarcoplasmic reticulum refilling and unloading. *Br. J. Pharmacol.*, **109**, 336–343.

DARBY, P.J., KWAN, C-Y. & DANIEL, E.E. (1993). Use of calcium pump inhibitors in the study of calcium regulation in smooth muscle. *Biol. Signals*, **2**, 293–304.

FASOLATO, C., HOTH, M & PENNER, R. (1993). A GTP-dependent step in the activation mechanism of capacitative calcium influx. *J. Biol. Chem.*, **268**, 20737–20740.

GOMES DE COSTA, A.G. & MADEIRA, V.M.C. (1986). Magnesium and manganese ions modulate Ca^{2+} uptake and its energic coupling in sarcoplasmic reticulum. *Arch. Biochem. Biophys.*, **249**, 199–206.

HASHIMOTO, T., HIRATA, M., ITOH, T., KANMURA, Y. & KURIYAMA, H. (1986). Inositol 1,4,5-trisphosphate activates pharmacomechanical coupling in smooth muscle of the rabbit mesenteric artery. *J. Physiol.*, **370**, 605–618.

HOTH, M. & PENNER, R. (1992). Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature*, **355**, 353–356.

IINO, M. (1989). Calcium-induced calcium release mechanism in guinea pig taenia caeci. *J. Gen. Physiol.*, **94**, 368–383.

ITO, S., OHTA, T. & NAKAZATO, Y. (1993). Inward current activated by carbachol in rat intestinal smooth muscle cells. *J. Physiol.*, **470**, 395–409.

JACOB, R. (1990). Agonist-stimulated divalent cation entry into single cultured human umbilical vein endothelial cells. *J. Physiol.*, **421**, 55–77.

KASAI, Y., IINO, M., TSUTSUMI, O., TAKETANI, Y. & ENDO, M. (1994). Effects of cyclopiazonic acid on rhythmic contractions in uterine smooth muscle bundles of the rat. *Br. J. Pharmacol.*, **112**, 1132–1136.

MISSIAEN, L., DECLERCK, I., DROOGMANS, G., PLESSERS, L., DE SMEDT, H., RAEYMAEKERS, L. & CASTEELS, R. (1990). Agonist-dependent Ca^{2+} and Mn^{2+} entry dependent on state of filling of Ca^{2+} stores in aortic smooth muscle cells of the rat. *J. Physiol.*, **427**, 171–186.

MUNRO, D.D. & WENDT, I.R. (1994). Effects of cyclopiazonic acid on $[Ca^{2+}]_i$ and contraction in rat urinary bladder smooth muscle. *Cell Calcium*, **15**, 369–380.

NOGUERA, M.A. & D'OCON, M.P. (1993). Evidence that depletion of internal calcium stores sensitive to noradrenaline elicits a contractile response dependent on extracellular calcium in rat aorta. *Br. J. Pharmacol.*, **110**, 861–867.

OHTA, T., ITO, S. & NAKAZATO, Y. (1993). Chloride currents activated by caffeine in rat intestinal smooth muscle cells. *J. Physiol.*, **465**, 149–162.

OHTA, T., ITO, S. & NAKAZATO, Y. (1994). All-or-nothing responses to carbachol in single intestinal smooth muscle cells of rat. *Br. J. Pharmacol.*, **112**, 972–976.

PACAUD, P. & BOLTON, T.B. (1991). Relationship between muscarinic receptor cationic current and internal calcium in guinea-pig jejunal smooth muscle cells. *J. Physiol.*, **441**, 477–499.

PACAUD, P., LOIRAND, G., GRÉGOIRE, G., MIRONNEAU, C. & MIRONNEAU, J. (1993). Noradrenaline-activated heparin-sensitive Ca^{2+} entry after depletion of intracellular Ca^{2+} store in portal vein smooth muscle cells. *J. Biol. Chem.*, **268**, 3866–3872.

PAREKH, A.B., TERLAU, H. & STUHMER, W. (1993). Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. *Nature*, **364**, 814–818.

PUTNEY, J.W. Jr. (1986). A model for receptor-regulated calcium entry. *Cell Calcium*, **7**, 1–12.

PUTNEY, J.W. Jr. (1990). Capacitative calcium entry revisited. *Cell Calcium*, **11**, 611–624.

RANDRIAMAMPITA, C. & TSIEN, R.Y. (1993). Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. *Nature*, **364**, 809–814.

SEIDLER N.M., JONA, I., VEGH, M. & MARTONOSI, A. (1989). Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J. Biol. Chem.*, **264**, 17816–17823.

SHIMA, H. & BLAUSTEIN, M.P. (1992). Modulation of evoked contractions in rat arteries by ryanodine, thapsigargin and cyclopiazonic acid. *Circ. Res.*, **70**, 968–977.

SJODIN, R.A. (1959). Rubidium and cesium fluxs in muscle as related to the membrane potential. *J. Gen. Physiol.*, **42**, 983–1003.

SOMLYO, A.V., BOND, M., SOMLYO, A.P. & SCARPA, A. (1985). Inositol trisphosphate-induced calcium release and contraction in vascular smooth muscle. *Proc. Natl. Acad. Sci. USA*, **85**, 5231–5235.

UYAMA, Y., IMAIZUMI, Y. & WATANABA, M. (1992). Effects of cyclopiazonic acid, a novel Ca^{2+}-ATPase inhibitor, on contractile responses in skinned ileal smooth muscle. *Br. J. Pharmacol.*, **106**, 208–214.

XUAN, Y-T., WANG O-L. & WHORTON, A.R. (1992). Thapsigargin stimulates Ca^{2+} entry in vascular smooth muscle cells: nicardipine-sensitive and -insensitive pathways. *Am. J. Physiol.*, **262**, C1258–C1265.

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