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EFFECTS OF CHLORPROMAZINE ON THE ELECTRICAL AND MECHANICAL PROPERTIES OF INTACT AND SKINNED MUSCLE CELLS OF GUINEA-PIG MESENTERIC ARTERY

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1 Effects of chlorpromazine on the contraction evoked in intact muscles, and of chlorpromazine or calmodulin on the contraction evoked in the saponin-treated skinned muscles of the guinea-pig mesenteric artery were investigated.

2 Chlorpromazine, above 5×10^{-7} M, depolarized the membrane and reduced the membrane resistance measured from the current-voltage relationships at the depolarized and resting membrane potential levels.

3 The contraction evoked by excess $[K]_o$, NaCl-free Krebs and by caffeine was suppressed by application of chlorpromazine; contractions induced by the former two treatments were suppressed to a greater extent than those induced by the latter. Contractions induced by excess $[K]_o$ or NaCl-free Krebs ceased in Ca-free solution, but not those induced by caffeine, i.e. influxes of Ca across the membrane were more suppressed than was the release of Ca from the storage sites by chlorpromazine.

4 In skinned muscles, chlorpromazine suppressed and calmodulin enhanced the Ca-induced contraction in a dose-dependent fashion. The minimum concentration of chlorpromazine required to suppress the Ca-induced contraction (10^{-5} M) was 10^{-6} M and that of calmodulin to enhance the Ca-induced contraction was 10^{-7} M.

5 After skinned muscles had been loaded with Ca, chlorpromazine or calmodulin itself did not induce the contraction by the release of stored Ca in the cell. However, calmodulin increased and chlorpromazine suppressed the accumulation of Ca into the storage site (presumably sarcoplasmic reticulum).

6 From the present results, it is suggested that chlorpromazine acts on the surface membrane and suppresses Ca-influx, thus causing a relaxation of the mesenteric artery. However, when chlorpromazine or calmodulin was applied to skinned muscles, the former suppresses and the latter enhances the Ca accumulation into the storage site and the activation of contractile proteins. Thus chlorpromazine causes the relaxation of the vascular tissue. However, a lower concentration of chlorpromazine was required to suppress the Ca influx than to suppress the Ca accumulation into the storage site or the activity of calmodulin in contractile protein. Thus vasodilatation induced by chlorpromazine *in vitro*, is mainly due to the suppression of Ca influx.

Introduction

Calmodulin (Cheung, 1970; Kakiuchi & Yamazaki, 1970) plays a role as a Ca regulator protein in visceral smooth muscles (Hartshorne, 1980; Adelstein & Eisenberg, 1980). In skinned muscles, cyclic adenosine 3′,5′-monophosphate (cyclic AMP)-dependent protein kinase and calmodulin also regulate muscle contraction (Sparrow, Mrwa, Hofman & Rüegg, 1981; Kerrick, Hoar, Cassidy, Bolles & Malencik, 1981). In vascular tissues, calmodulin inhibitors also suppress the activity of Ca-dependent adenosine triphosphatase (ATPase) (Hidaka, Yamaki, Naka, Tanaka, Hayashi & Kobayashi, 1980; Asano, Suzuki & Hidaka, 1981).

Chlorpromazine, a phenothiazine derivative, competes with calmodulin, the Ca-receptor of the contractile protein, in various tissues (Honda & Imamura, 1968; Weiss, Fertel, Figlin & Uzunov, 1974; Brostrom, Huang, Breckenridge & Wolff, 1975; Levin & Weiss, 1976; 1977; 1979; Hidaka, Asano, Iwadare, Matsumoto, Totsuka & Aoki, 1978; Norman & Drummond, 1979; Means & Dedman, 1980; Asano *et al.*, 1981).

Responses to exogeneously applied drugs in intact muscles cells, skinned muscles, or biochemically prepared organelles are not always the same, because functional links between myoplasmic membranes

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and internal organelles in the physiological environment are not measurable in the latter two conditions; in addition the ionic permeability of the myoplasmic membrane to many drugs has not yet been measured.

The present experiments were carried out to investigate the effects of chlorpromazine on intact muscles and to determine the effects of this agent and of calmodulin on saponin-treated skinned muscles (Saida & Nonomura, 1978; Itoh, Kajiwara, Kitamura & Kuriyama, 1981a; Itoh, Kuriyama & Suzuki, 1981b; Itoh, Suzuki & Kuriyama, 1981c). The results showed that chlorpromazine suppressed the contraction evoked in intact muscles, suppressed the accumulation of Ca into the storage site and Ca receptors in contractile proteins in skinned muscles. Exogenously applied calmodulin enhanced the accumulation of Ca into the storage site and the sensitivity of Ca-receptors in the contractile protein. As a result this agent antagonized the action of chlorpromazine. However, in intact and skinned muscle cells, a lower concentration of chlorpromazine was required to suppress the Ca influx than to suppress the accumulation of Ca into the storage site or the activity of calmodulin in the contractile protein. Chlorpromazine may act as a relaxant in vascular smooth muscle by suppressing the Ca-influx.

Methods

Albino guinea-pigs of either sex (300–400 g) were stunned and bled. The mesenteric vascular bed was placed in a dissecting chamber filled with Krebs solution, and the mesenteric artery was dissected.

In the microelectrode experiments, the dissected artery was mounted in an organ bath without the removal of surrounding tissues such as the mesenteric vein, mesenteric membrane and lymphatic vessels. The organ bath had a capacity of approx. 2 ml and warmed Krebs solution (34–35°C) was superfused at a flow rate of about 3 ml/min.

Ionic composition of the Krebs solution was as follows (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5. High-$[K]_o$ solution or NaCl-free solution was prepared by replacing NaCl with KCl or choline-Cl (containing atropine 1 μg/ml), respectively.

To stimulate the muscle, the partition stimulating method was used (Abe & Tomita, 1968). The current pulse was applied to the long axis of the vessel. A glass capillary microelectrode filled with 3 M KCl and with a tip resistance of 40–80 MΩ was used to impale single cells and the electrical activities of the muscle membrane were recorded (Nihon Kohden Recticorder; RJG 4024).

Mechanical responses of the artery were measured by attaching a circular strip of the mesenteric artery (100 μm wide and 300 μm long) to a strain gauge (U-gauge, Shiko Seiki). The tissue was superfused in an organ bath (0.9 ml capacity) filled with Krebs solution. Solutions containing drugs or modified ionic concentrations were added to the bath while the solution already present was sucked off with an aspirator; therefore only a few seconds were needed to apply the test solutions. However, this procedure was accompanied by large artifacts due to the sudden change in the level of the solution in the organ bath. To correct this, before the experiment, recovery of the position of the recording pen to the original level was checked, and the recording systems were adjusted appropriately. Chlorpromazine was added for 10 min before the application of various stimulants.

Skinned muscle preparations were obtained by using saponin, according to the method described by Itoh *et al.* (1981b). After a K-induced contraction was recorded from the intact muscle, the bathing solution was replaced with a relaxing solution containing 130 mM KCl, 20 mM Tris-maleate, 5 mM $MgCl_2$, 5 mM ATP (10 mM Na as Na_2 ATP) and 4 mM EGTA at pH 6.8. The preparation remained for 20 min in the relaxing solution containing 50 μg/ml saponin (ICN). The preparation was washed again with the same solution and left until the tension level became constant at about zero. Immediately before application of a Ca-containing solution, the preparation was superfused with the relaxing solution. These procedures were similar to those described by Saida & Nonomura (1978). To investigate the effects of chlorpromazine on the Ca-receptor of contractile proteins, the Ca-tension-relation curve in the presence or absence of chlorpromazine was observed. This agent was applied during the last 10 min of saponin treatment and Ca was applied cumulatively. Various Ca concentrations were prepared by adding appropriate amounts of $CaCl_2$ to EGTA. The apparent binding constant of EGTA for Ca was considered to be 10^6 M^{-1} at pH 6.8 and 25°C (Itoh *et al.*, 1981b).

To obtain a caffeine-induced contraction, the concentration of EGTA in the relaxing solution was reduced to 10^{-4} M throughout the experiment. The pH of the relaxing and of the various Ca solutions was maintained at 6.8 by addition of KOH instead of KCl, isotonically.

Chemicals used in the experiments were atropine sulphate (Daiichi), caffeine (Wako), saponin (ICN), chlorpromazine (Sigma) and EGTA (Dozin). These drug solutions were freshly prepared before each experiment.

Observed experimental values were expressed by mean ± s.d. Statistical significance was assessed by Student's *t* test, and probabilities of less than 5% were considered to be significant.

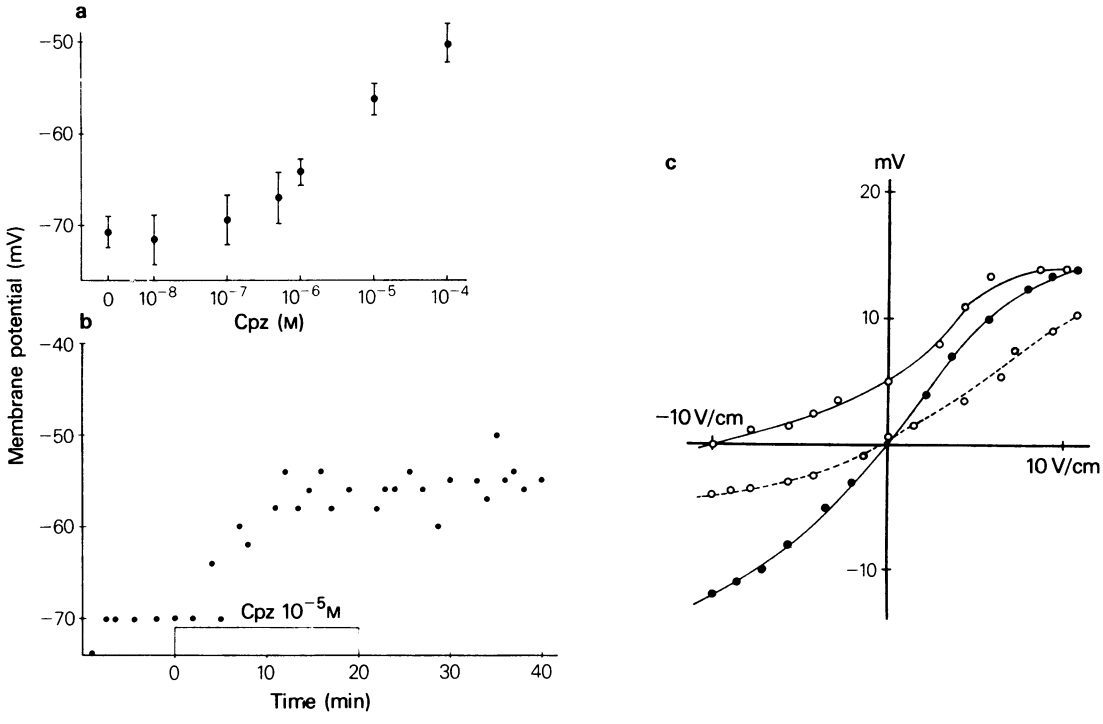

Figure 1 Effects of chlorpromazine on membrane potential and membrane resistance in the guinea-pig mesenteric artery. (a) The relationship between membrane potential and chlorpromazine (Cpz) concentration. Membrane potentials were measured 10 to 20 min after application of chlorpromazine. Mean values are shown; vertical lines indicate s.d. (b) Changes in the membrane potential induced by 10^{-5} M chlorpromazine (20 min). Membrane potentials were measured by successive impalements of different cells by the microelectrode. (c) Current-voltage relationship obtained before (●) and during application of chlorpromazine 10^{-6} M (○). Duration of current pulses was 1.5 s. In the presence of chlorpromazine (10–20 min after application), the membrane potential was displaced to the level of resting membrane potential by application of inward current, and the current-voltage relationship was measured by application of various intensities of inward and outward current pulses (dotted line). The microelectrode was inserted into the same cell throughout the experiment at 0.05 mm distance from the stimulating electrode.

Results

Effects of chlorpromazine on the intact muscles

The membrane potential of smooth muscle cells of the guinea-pig mesenteric artery was -70.4 ± 1.4 mV ($n = 30$). Chlorpromazine in concentrations over 10^{-6} M depolarized the membrane to -64.8 ± 0.8 mV, $n = 15$, $P < 0.05$, and in 10^{-4} M, to -51.2 ± 2.1 mV, $n = 15$, $P < 0.001$ (Figure 1a). When a low concentration of chlorpromazine was applied, the change in membrane potential was reversible, but above 10^{-6} M, depolarization was irreversible. Figure 1b shows the effects of 10^{-5} M chlorpromazine on the membrane potential. To measure the membrane potential, the microelectrode was inserted repeatedly into the cells. Depolarizations of the membrane started a few minutes after the Krebs solution was replaced with the chlorpromazine-containing solution and the depolarization was sustained after the tissue was rinsed with Krebs solution. Even when the superfusate was replaced within 1 min, the change of the membrane potential appeared after a certain latency.

Figure 1c shows the effects of 10^{-6} M chlorpromazine on the current-voltage relationship. The microelectrode was inserted into the same cell throughout the experiment at a distance of 0.05 mm from the stimulating electrode. Chlorpromazine depolarized the membrane and the current-voltage relation curve showed a reduction in the membrane resistance. To determine more precisely changes in the membrane resistance, the membrane depolarized by chlorpromazine was displaced to the level of the resting membrane potential by application of inward currents, and the current-voltage relation was measured. The membrane resistance measured by inward and outward current pulses was con-

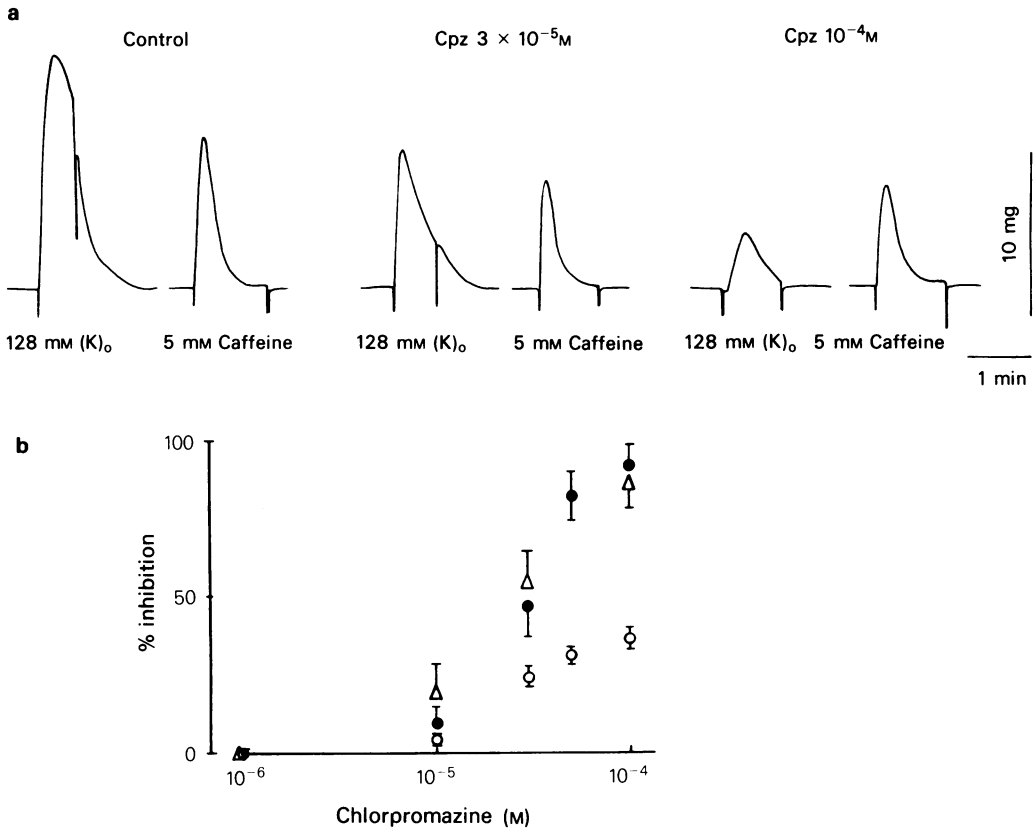

Figure 2 (a) Effects of chlorpromazine (Cpz; 3×10^{-5} M and 10^{-4} M) on the K-induced and caffeine-induced contraction of the guinea-pig mesenteric artery. (b) The inhibitory effect of chlorpromazine (absscissa scale) on the contraction of the guinea-pig mesenteric artery produced by 128 mM $[K]_o$ (●); NaCl-free solution (choline-Cl with atropine 1 μg/ml) (△) and 5 mM caffeine (○). Chlorpromazine was added 10 min before application of the various stimulants. Vertical bars represent s.d. or 2 × s.d. ($n = 5-6$).

sistently reduced. These results indicate that depolarizations induced by chlorpromazine are due to an increase in the ionic conductance of the membrane.

The effects of chlorpromazine on the mechanical response evoked by excess $[K]_o$, NaCl-free Krebs solution or caffeine were then examined. As shown in Figure 2a, chlorpromazine ($> 10^{-5}$ M) suppressed the contraction evoked by excess $[K]_o$ or caffeine. The K-induced and NaCl free-induced contractions were suppressed by chlorpromazine to a greater extent than were those induced by caffeine. The relationships between the inhibition of the contraction evoked by excess $[K]_o$, NaCl-free Krebs solution or caffeine and different concentrations of chlorpromazine were plotted in Figure 2b. The inhibitory effect is more pronounced on the contraction evoked by 128 mM $[K]_o$ or NaCl-free solution than on that induced by 5 mM caffeine. In the guinea-pig mesenteric artery, the contraction evoked by excess $[K]_o$ or NaCl-free Krebs solution, but not that induced by caffeine, was abolished in Ca-free EGTA (2 mM) containing solution. The contraction induced by caffeine was mainly due to the release of Ca stored in the cell (Itoh *et al.*, 1981b). Furthermore, diltiazem, a Ca channel blocker, suppressed the contraction evoked by excess $[K]_o$ but not that evoked by NaCl-free solution (Itoh *et al.*, 1981c; Suzuki, Itoh & Kuriyama, 1981). These results indicate that chlorpromazine suppressed to a greater extent, the contraction evoked by influx of Ca than that caused by the release of Ca from the storage site, and that this action of chlorpromazine differs from that of a Ca channel blocker.

The effects of chlorpromazine on the contraction evoked by various concentrations of $[K]_o$ were also investigated. As shown in Figure 3, application of 10^{-5} M chlorpromazine consistently suppressed the K-induced contraction in concentrations ranging be-

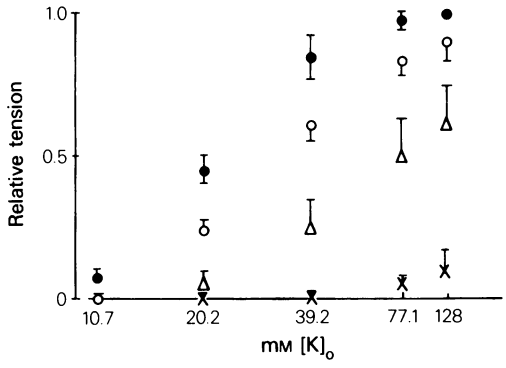

Figure 3 Effects of chlorpromazine on the K-induced contraction observed before (●) and during (○: 10^{-5} M, △: 3×10^{-5} M, X: 10^{-4} M) application of chlorpromazine. Each value is expressed as a relaxation of the contraction induced by 128 mM $[K]_o$ in the absence of chlorpromazine. Vertical bars represents s.d. or 2 × s.d. ($n = 4-6$).

tween 10.7 mM and 128 mM $[K]_o$, and in the presence of 10^{-4} M chlorpromazine, the contraction evoked by all ranges of $[K]_o$ was all but abolished.

Effects of calmodulin and chlorpromazine on skinned muscles

As shown in Figure 4, before preparing skinned muscles, the amplitude of the contraction evoked by 128 mM $[K]_o$ in intact cells was recorded. This served as an indication of the completion of skinning in that the maximum amplitude of contraction produced by 10^{-5} M Ca was larger than that of 128 mM $[K]_o$-induced contraction in the intact cells (Itoh *et al.*, 1981b). The intact tissue was then skinned by rinsing it with the relaxing solution and applying saponin (50 μg/ml) for about 20 min, as described in methods. Application of 10^{-5} M Ca produced a contraction, the amplitude of which was larger than that evoked by 128 mM $[K]_o$ in intact muscles (Figure 4a and b). After the Ca-induced contraction had reached a steady amplitude, three different concentrations of calmodulin (10^{-7} M–5×10^{-7} M) were applied. The amplitude of the Ca-induced contraction was increased by application of 10^{-7} M calmodulin. Figure 5 shows the effects of various concentrations of chlorpromazine (10^{-6} M–10^{-4} M) on the Ca-induced contraction (10^{-5} M Ca). The contraction evoked by 128 mM $[K]_o$ was recorded from the intact muscles (Figure 5a(i)). After the Ca-induced contraction had reached a steady amplitude in the skinned muscles, chlorpromazine was applied (Figure 5a(ii)). The relationship between the inhibition induced by chlorpromazine and the concentration of chlorpromazine is shown in Figure 5b. In the presence of chlorpromazine, the contraction evoked by 10^{-5} M Ca was suppressed consistently in the skinned muscles.

The free Ca-tension relationships of skinned muscles in the presence of calmodulin or chlorpromazine

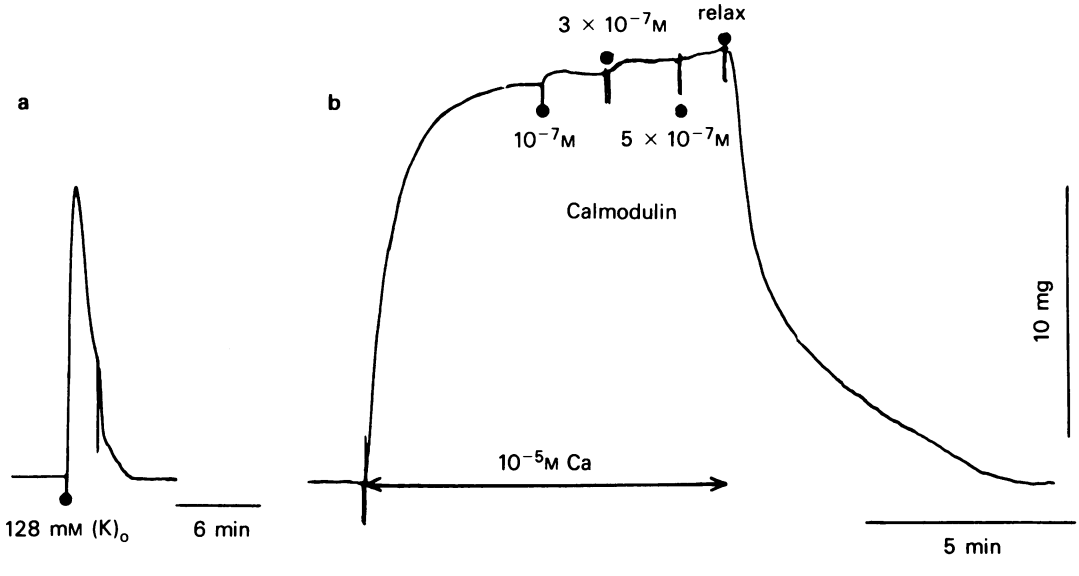

Figure 4 Effects of calmodulin on the Ca-induced contraction in skinned muscles. (a) Contraction evoked by 128 mM $[K]_o$ solution (applied at ●) before treatment with saponin. (b) In skinned muscle, the 10^{-5} M Ca-induced contraction was much larger than that evoked by 128 mM $[K]_o$ in the intact muscle. The contraction evoked by 10^{-5} M Ca was enhanced in a dose-related manner by application of 10^{-7} M to 5×10^{-7} M calmodulin. Dots indicate cumulative applications of calmodulin and wash (relax).

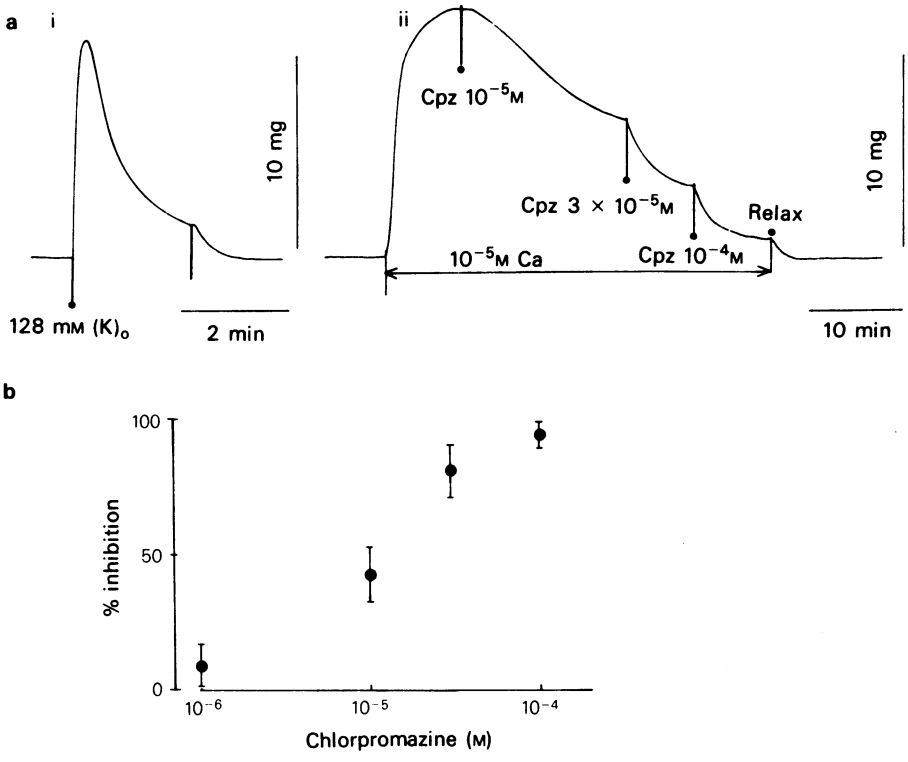

Figure 5 Effects of chlorpromazine (Cpz) on the Ca-induced contraction in skinned muscles. (a)(i): Contraction evoked by 128 mM $[K]_o$ solution in intact muscle. (ii) In skinned muscle, 10^{-5} M Ca-induced contraction was suppressed in a dose-related manner by application of 10^{-5} M to 10^{-4} M chlorpromazine. (b) The relaxation produced by different doses of chlorpromazine on the contraction evoked by 10^{-5} M Ca in skinned muscles. Chlorpromazine was applied in the cumulative manner shown in (a ii). Vertical bars represent 2 × s.d. ($n = 4$).

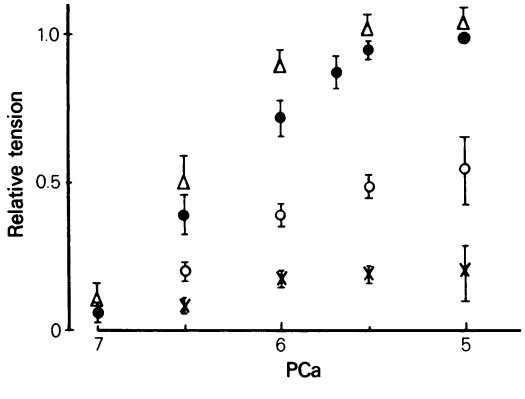

Figure 6 Effects of calmodulin or chlorpromazine on the free Ca (PCa)-tension relationship in skinned muscles. The amplitude of the contraction induced by 10^{-5} M Ca is registered as a relative tension of 1.0. (△) Calmodulin; (●) control; (○) 3×10^{-5} M chlorpromazine; (×) 10^{-4} M chlorpromazine. Vertical bars indicate s.d. of mean or 2 × s.d. ($n = 5-6$).

are summarized in Figure 6. The minimum concentration of Ca required to generate the contraction was just over of 10^{-7} M and the maximum contraction was recorded by application of 10^{-5} M Ca. This finding confirmed previous observations made in skinned smooth muscles (Itoh *et al.*, 1981b). Application of calmodulin (10^{-7} M) increased the amplitude of the Ca-induced contraction at any given concentration of Ca (10^{-7} M–10^{-5} M). The enhancement of the Ca-induced contraction was observed at low Ca concentrations in the presence of 10^{-7} M calmodulin. Application of chlorpromazine (3×10^{-5} M) consistently suppressed the Ca-induced contraction at each concentration of Ca investigated.

Interactions between calmodulin and chlorpromazine on the Ca-induced contraction were then investigated. In skinned muscles, the mechanical response was evoked by application of 10^{-5} M Ca. Chlorpromazine reduced the amplitude of contraction in a dose-dependent fashion and calmodulin restored the amplitude of contraction also in a dose-dependent fashion. For example (Figure 7), the re-

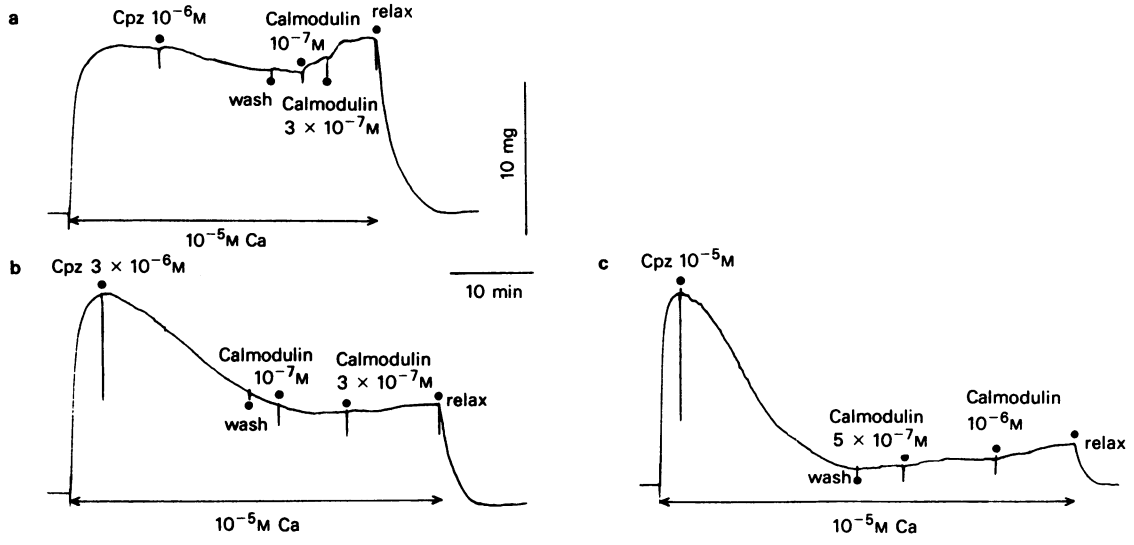

Figure 7 Effects of chlorpromazine (Cpz) and exogenously applied calmodulin on the Ca-induced contraction in the guinea-pig mesenteric artery. (a) The 10^{-5} M Ca-induced contraction (max. tension) was suppressed slightly by 10^{-6} M chlorpromazine, restored by 10^{-7} M and enhanced by 3×10^{-7} M calmodulin. (b) 10^{-5} M Ca-induced contraction was suppressed to a greater extent than before by (a) by 3×10^{-6} M chlorpromazine. This suppression was not restored by 10^{-7} M calmodulin but was prevented from increasing by application of 3×10^{-7} M calmodulin. (c) 10^{-5} M Ca-induced contraction was suppressed almost totally by 10^{-6} M chlorpromazine. This suppression was not restored by application of 10^{-6} M calmodulin.

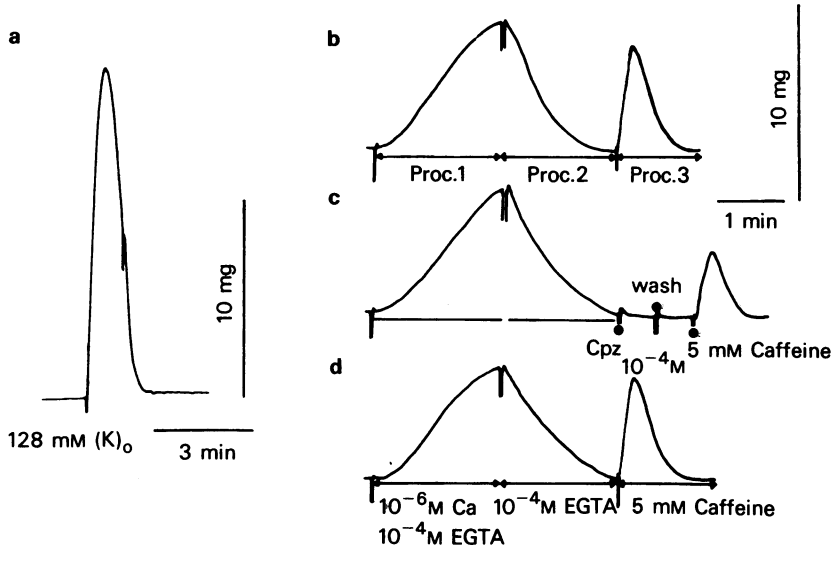

Figure 8 Effects of caffeine or chlorpromazine (Cpz) in the relaxing solution in skinned muscle cells following Ca loading. (a) Effects of 128 mM $[K]_o$ on intact cells; (b–d) Proc. 1: 10^{-6} M Ca with 10^{-4} M EGTA containing solution was applied from 2 min. Proc. 2: the tissue was rinsed with 10^{-4} M EGTA containing relaxing solution for 2 min. Proc. 3: 5 mM caffeine was applied. In (c) during proc. 3, chlorpromazine was added to the relaxing solution. In the same records, 5 mM caffeine was subsequently applied to the tissue.

duction in the amplitude of contraction induced by 10^{-6} M chlorpromazine was restored completely by 10^{-7} M calmodulin and an increased concentration of calmodulin (3×10^{-7} M) enhanced the amplitude of the Ca-induced contraction over the control levels (Figure 7a). In the presence of higher concentrations of chlorpromazine, higher concentrations of calmodulin were required (Figure 7b and c). These results suggest that application of chlorpromazine reduces the amplitude of the Ca-induced contraction dose-dependently and calmodulin acts competitively on the inhibitory action of chlorpromazine on the Ca-induced contraction.

To investigate the effects of chlorpromazine on the Ca accumulating and releasing sites, its effects on caffeine-induced contractions in skinned muscles were investigated on the assumption that the amplitude of caffeine-induced contraction indicates the amount of Ca stored in the cell.

To evoke the caffeine-induced contractions in skinned muscles the following procedures were adopted: 10^{-6} M Ca with 10^{-4} M EGTA were applied for 2 min or 3 min (Ca accumulation into the storage site; procedure 1), and subsequent contractions were evoked by application of 5 mM caffeine (Ca release; procedure 3) following a period of 2 min wash in Ca-free 10^{-4} M EGTA containing solution (removal of free Ca; procedure 2) (Itoh *et al.*, 1981b). The schematic arrangement is shown in Figure 8b. Before preparing the skinned muscle, the 128 mM $[K]_o$-induced contraction was recorded as an indicator of completeness of skinning (Figure 8a). Application of caffeine (5 mM) produced the contraction during procedure 3, but 10^{-4} M chlorpromazine (c) or 10^{-7} M calmodulin did not. The amplitude of the caffeine-induced contraction was reduced by chlorpromazine. This effect was due, in part, to the suppression of the Ca-receptor of the contractile protein, as observed from the effects of chlorpromazine on the Ca-tension relationship, and also in part to a suppression of the release of Ca stored in the cell.

Figure 9 shows the effects of calmodulin and chlorpromazine on the caffeine-induced contraction in skinned muscles. When 10^{-7} M calmodulin was applied during procedure 1, the subsequent caffeine-induced contraction increased in amplitude ($113.1 \pm 7.1\%$, $n = 6$) compared with that obtained in the absence of calmodulin. Application of calmodulin during procedures 2 and 3 had negligible effects. This indicates that calmodulin affects mainly the Ca accumulation into the storage sites. To observe the effects of chlorpromazine on the caffeine-induced contraction, the tissue were preincubated with 10^{-6} M Ca containing 10^{-4} M EGTA for 3 min. This 1 min prolongation of the Ca-incubation time was because of the slow appearance of the effects of chlorpromazine on the skinned muscles. Chlorpromazine suppressed the amplitude of the caffeine-induced contraction particularly when this agent was applied during procedure 1 ($52.6 \pm 5.1\%$ of the control observed in the absence of chlorpromazine, $n = 7$ in Figure 9b(ii)). However, this inhibition was not due solely to the suppression of Ca release from the storage site by inhibition of the accumulation of Ca, but was also due to suppression of the Ca receptor of the contractile proteins. Thus application of chlorpromazine during procedures 2 and 3, reduced the amplitude of caffeine-induced contraction to $72.8 \pm 4.4\%$ ($n = 8$) of the control value.

Discussion

In the guinea-pig mesenteric artery, the contraction evoked by excess $[K]_o$ or by NaCl-free solution ceased in Ca-free solution. The contraction evoked by excess $[K]_o$ was suppressed by application of diltiazem, a Ca channel blocker, while that evoked by NaCl-free solution was not (Itoh *et al.*, 1981c; Suzuki *et al.*, 1981). Chlorpromazine suppressed both these contractions, but that induced by caffeine which was generated by the release of Ca from the storage site was inhibited only slightly. The extent of the suppression of the K-induced contraction in intact muscles and that of the Ca-induced contraction in skinned muscles by chlorpromazine was similar but that of the caffeine contraction in intact muscles was not. This implies that in intact muscles, chlorpromazine acts mainly on the surface membrane to suppress the Ca influx, and as a consequence, the amplitude of the contraction is suppressed.

During application of chlorpromazine, the membrane resistance was markedly reduced and the membrane depolarized. The equilibrium potentials calculated from the Nernst equation for Na, Ca and Cl ions (E_{Na}, E_{Ca} and E_{Cl}) in vascular muscles are thought to be lower than the resting membrane potential (Casteels, 1981; Jones, 1981). The influx of Ca was suppressed during application of chlorpromazine; thus, the increased ionic conductance of the membrane is presumably due to an increase in the Na- and/or Cl-permeability. Chlorpromazine acts as a calmodulin inhibitor in various tissues (Levin & Weiss, 1976; 1979; Hidaka *et al.*, 1978; 1980; Hidaka, Sasaki, Tanaka, Endo, Ohno, Fujii & Nagata, 1981). Therefore, if the control of Ca permeability at the surface membrane (myoplasmic membrane) requires the presence of calmodulin, as is the case for the Ca pump at the red cell membrane (Marx, 1980), chlorpromazine may act on the Ca-permeability at the surface membrane though this effect may not be confined to the calmodulin of the myosin light chain phosphokinase in the cell (Hidaka *et al.*, 1980). In the present experiments, the relationship between the

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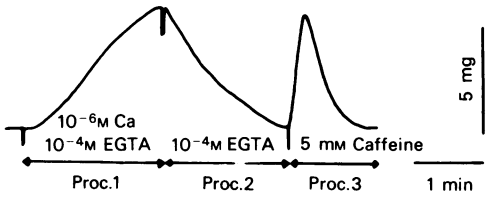


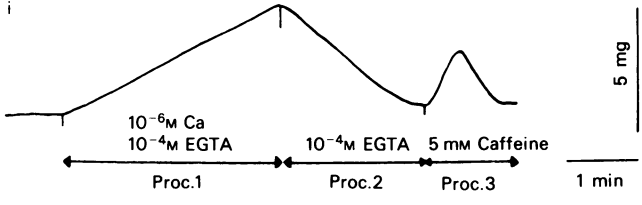
ii

Effect of calmodulin on the caffeine-induced contraction in skinned muscles. Calmodulin 10^{-7}M was applied as shown in the above scheme.

Application of 10^{-7}M calmodulin	% change (control: 100) mean ± s.d.
Proc.1	113.1±7.1 (*n*:6)
Proc.2	101.8±4.7 (*n*:4)
Proc.3	103.9±2.7 (*n*:6)
Proc.1+2+3	119.8±3.5 (*n*:5)

b

i



ii

Effect of chlorpromazine on the caffeine-induced contraction in skinned muscles. Chlorpromazine 10^{-4}M was applied as shown in the above scheme.

Application 10^{-4}M chlorpromazine	% change (control: 100) mean ± s.d.
Proc.1	52.6±5.1 (*n*:7)
Proc.2+3	72.8±4.4 (*n*:8)
Proc.1+2+3	47.2±8.0 (*n*:9)

Figure 9 (a) Effects of calmodulin (10^{-7}M) on the accumulation into or release of Ca from the storage sites in skinned muscles. (i) Schematic diagram of procedure (Proc). Proc. 1: 10^{-6}M Ca with 10^{-4}M EGTA containing solution was applied for 2 min. Proc. 2: the tissue was rinsed with 10^{-4}M EGTA containing relaxing solution for 2 min. Proc. 3: 5 mM caffeine was applied. (ii) Effects of calmodulin (10^{-7}M) on the accumulation into or release of Ca from storage sites estimated from the amplitude of caffeine-induced contraction. The amplitude of 5 mM caffeine-induced contractions in the absence of calmodulin was assigned a relative tension of 100%. (b) Effects of chlorpromazine (10^{-4}M) on the accumulation into or release of Ca from the storage sites in skinned muscles. (i) Schematic diagram. Proc. 1: 10^{-6}M Ca with 10^{-4}M EGTA containing solution was applied for 3 min. Proc. 2: 10^{-4}M containing relaxing solution for 2 min. Proc. 3: 5 mM caffeine application. (ii) Effects of chlorpromazine (10^{-4}M) on the caffeine-induced contraction. The amplitude of 5 mM caffeine-induced contraction observed in the absence of chlorpromazine was assigned a relative tension of 100%.

suppression of the Ca-influx and the increase of Na-permeability was not elucidated. The contraction produced by voltage-dependent Ca influx was selectively suppressed by diltiazem, a Ca channel blocker (Suzuki *et al.*, 1981) i.e. diltiazem suppressed the K-induced but not the NaCl-free solution-induced contraction. These two contractions were suppressed by chlorpromazine. Therefore, the suppression of Ca-influx in the presence of chlorpromazine was non-selective. Furthermore, chlorpromazine-induced depolarization was irreversible; therefore, this agent may not be selective for calmodulin. In the rabbit aorta, chlorpromazine (10^{-6} M) antagonized contractions produced by noradrenaline, 5-hydroxytryptamine and histamine, but not those produced by prostaglandin $F_{2\alpha}$ and angiotensin II (Asano *et al.*, 1981). If the latter two substances release the stored Ca as in the case of caffeine, the present experiments show that chlorpromazine did not suppress the contraction.

The membrane and contractile properties of vascular smooth muscles differs with the region and species, e.g., in the case of the mesenteric artery, the Ca stored in the cell was released almost completely by application of caffeine, but in the porcine coronary artery, only part of the stored Ca was released by caffeine (Itoh *et al.*, 1981a,b). A systematic examination is required to compare the results obtained in different vascular tissues.

In the guinea-pig mesenteric artery, calmodulin accelerated Ca accumulation into the storage site by activation of the Ca-pump (Marx, 1980) but the release of Ca remained unaffected. Furthermore, calmodulin enhanced but chlorpromazine suppressed the amplitude of the Ca-induced contraction. Exogeneously or endogenously applied, calmodulin may act on the light chain phosphokinase of myosin (Sparrow *et al.*, 1981; Vallet, Molla & Demaille, 1981). On the other hand, chlorpromazine suppressed these calmodulin actions, and relaxation of the tissue followed. It is still uncertain whether chlorpromazine actually penetrates the cell.

Using biochemical procedures (Honda & Imamura, 1968; Weiss *et al.*, 1974; Brostrom *et al.*, 1975; Hidaka *et al.*, 1978; Levin & Weiss, 1979; Asano *et al.*, 1981) the effects of chlorpromazine have been clarified in relation to the calmodulin actions on the light chain phosphokinase of myosin in smooth muscles and in other organelles. However, the concentration of chlorpromazine required to suppress the Ca influx is much less than that required to affect contraction in intact skinned muscles.

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