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Effects of cyclopiazonic acid on contraction and intracellular Ca^{2+} in oesophageal striated muscle of normotensive and spontaneously hypertensive rats

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1 The effects of cyclopiazonic acid (CPA), a selective inhibitor of sarcoplasmic reticulum (SR) Ca^{2+}-ATPase, on twitch contraction and on the resting state of tension and intracellular Ca^{2+} level ($[Ca^{2+}]_i$) of the oesophageal striated muscle of stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive Wistar Kyoto rats (WKY) were compared.

2 CPA (10 μM) augmented the twitch contraction of oesophageal striated muscle preparations from both SHRSP and WKY, reducing the rate of relaxation ($-dT/dt$), and thus resulting in the prolongation of the time to 80% relaxation. The effect was significantly smaller in the SHRSP preparations.

3 In the resting state, CPA caused a sustained elevation of $[Ca^{2+}]_i$. The elevation was greater in the WKY preparations. Tension development accompanied by the elevation was observed in WKY preparations, but not in SHRSP preparations.

4 The sustained elevation of $[Ca^{2+}]_i$ induced by CPA was eliminated by the removal of extracellular Ca^{2+}. Both the elevated $[Ca^{2+}]_i$ and tension in the preparations from WKY were reduced by flufenamic acid (100 μM), mefenamic acid (100 μM), lanthanum (La^{3+}, 100 μM), gadolinium (Gd^{3+}, 100 μM) and SK&F 96365 (100 μM) but not by verapamil (10 μM).

5 Thapsigargin (3 μM), another SR Ca^{2+}-ATPase inhibitor, produced similar effects on basal tension to those of CPA, although it reduced the amplitude of twitch contraction.

6 These results suggest that in the rat oesophageal striated muscle, (1) CPA extends the sequestrating time of Ca^{2+} into the SR, (2) CPA induces a Ca^{2+} influx mediated through verapamil-insensitive pathways, possibly nonselective cation channels, and (3) the mechanism of $[Ca^{2+}]_i$ modulation due to CPA-sensitive SR Ca^{2+}-ATPase is deteriorated in the oesophageal striated muscle from SHRSP as compared with WKY preparations.

Keywords: Stroke-prone spontaneously hypertensive rats; oesophageal striated muscle; cyclopiazonic acid; sarcoplasmic reticulum; twitch contraction; resting state; tension; intracellular Ca^{2+} level; Ca^{2+} influx

Abbreviations: CPA, cyclopiazonic acid; $[Ca^{2+}]_i$, intracellular Ca^{2+} level; E-C coupling, excitation-contraction coupling; EGTA, ethylene-glycol-*bis* (β-amino-ether) N,N,N′,N′-tetraacetic acid; Gd^{3+}, gadolinium; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulphonic acid; La^{3+}, lanthanum; SBP, systolic blood pressure; SHRSP, stroke-prone spontaneously hypertensive rat; SK&F 96365, 1-[β-[3-(4-Methoxyphenyl)propoxy]-4-methoxyphenethyl]-1H-imidazole; SR, sarcoplasmic reticulum; WKY, Wistar Kyoto rat

Introduction

In striated muscles, it is established that Ca^{2+}, which induces contraction of the muscle, is released from the sarcoplasmic reticulum (SR), and that the relaxation of the muscle is brought about by Ca^{2+} uptake into the SR (Somlyo *et al*., 1981; see Ebashi & Endo, 1968; Endo, 1977; Gillis, 1985; Ríos & Pizarro, 1991; Schneider, 1994). Thus, the investigation of SR Ca^{2+} uptake activity is important for the evaluation of the excitation-contraction (E-C) coupling of striated muscles. Cyclopiazonic acid (CPA), a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+}-ATPase of SR in skeletal (Goeger & Riley, 1989; Seidler *et al*., 1989; Kurebayashi & Ogawa, 1991), cardiac (Takahashi *et al*., 1995) and smooth muscles (Uyama *et al*., 1992). CPA has therefore been used as a pharmacological tool to study E–C coupling in these muscles.

We have recently studied the effect of CPA on carotid arterial smooth muscle from Wistar Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP), and found that the CPA-induced contraction was greater in preparations from SHRSP (Sekiguchi *et al*., 1996). The contraction was brought about by an influx of extracellular Ca^{2+}, and the Ca^{2+} influx was mediated through verapamil-sensitive L-type voltage-dependent Ca^{2+} channels and verapamil-insensitive pathways. The greater contraction in the SHRSP carotid artery was due mainly to an increase in Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels. Similar results have been shown in the femoral artery of spontaneously hypertensive rats (SHR) (Nomura *et al*., 1997).

It has been known that Ca^{2+} handling of vascular smooth muscle is altered in the preparations from hypertensive rats (see Kwan, 1985; 1989). Abnormality of Ca^{2+} handling has been reported not only in vascular smooth muscle but also in visceral smooth muscles (Kwan *et al*., 1982; see also Kwan, 1985).

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Deficit of muscular function in skeletal muscle of SHR has also been reported (Carlsen & Gray, 1987). The outer layer of oesophagus has been reported to be composed of striated muscle (Gruber, 1968). It is of interest to investigate the difference in the action of the drugs which act on the SR between the visceral striated muscle from normotensive and hypertensive animals. In the present experiments, we examined the effect of CPA on the oesophageal striated muscle and report here marked differences in the action of the drug on both the contraction and $[Ca^{2+}]_i$.

Methods

Animals

Male WKY and SHRSP were used in the present experiments. These rats were purchased from Shimizu Laboratory Supplies Co. Ltd. (Kyoto, Japan) at 5 weeks of age and maintained in our animal facility at 22°C, 60% humidity under a 12 h light–dark cycle. Animals were given free access to normal chow (Funabashi SP) and tap water. The systolic blood pressure (SBP) of these rats was measured by the tail-cuff method. Prior to the SBP measurement, the rats were warmed at 40°C for 10 min. This procedure was necessary to obtain constant stable values of blood pressure.

Preparations and solutions

Rats were killed by bleeding from the vena cava after being anaesthetized with CO_2 at the age of 16 weeks. The oesophagus was excised and placed in a modified Tyrode's solution of the following composition (mM): NaCl 137, KCl 5.4, $CaCl_2$ 2.0, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6, equilibrated with a gas mixture of 95% O_2 and 5% CO_2. A solution containing 142.4 mM K^+ (high-K^+ Tyrode's solution) was made by replacing NaCl (137.0 mM) with equimolar KCl. A Ca^{2+}-free Tyrode's solution was made by omitting $CaCl_2$ and adding 2 mM ethylene glycol-*bis*(β-amino-ether)N,N,N′,N′-tetraacetic acid (EGTA). When gadolinium (Gd^{3+}) or lanthanum (La^{3+}) was used, the buffer system of the solution was changed for 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid (HEPES) system by omitting $NaHCO_3$ and NaH_2PO_4 and adding 10 mM HEPES adjusted to pH 7.3 with NaOH. This solution was aerated with O_2 gas. Connective tissue and fat surrounding the oesophagus were removed in the modified Tyrode's solution under a microscope. The oesophagus was opened longitudinally, and then the tunica muscularis mucosa was removed. The outer layer (striated muscle layer) of the oesophagus was cut into strips (2 mm in width and 10 mm in length) along the longitudinal direction of the muscle fibres. One end of the preparation was tied with a silk thread to a plastic holder, and the other was tied with a tungsten wire of 30 μm diameter. The holder was immersed in an organ bath of 10 ml volume filled with the modified Tyrode's solution (37°C). The tungsten wire tied to one end of the preparation was connected to a force-transducer. The preparations were stretched to 120% of the *in situ* length, and equilibrated in modified Tyrode's solution for at least 60 min. Then, the preparations were subjected to high-K^+-induced contraction twice for 3 min with an interval of 60 min by changing the solution to high-K^+ Tyrode's solution. The following experiments were performed after these procedures.

The preparations from extensor digitorum longus were made by halving the muscle longitudinally after the excision. The preparations were mounted on the organ bath and subjected to the following experiment in the same manner as those in the preparations from oesophagus.

Measurement of twitch contractions

The oesophageal striated muscle preparations were stimulated electrically with an electronic stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan) through two platinum wires placed close to each side of the preparation. Electrical pulses (10 ms duration, 5 V) were applied at a constant interval of 60 s. The interval of the stimulation was determined taking the duration of the twitch contraction in the presence of CPA (500–600 msec) into consideration. The other parameters were determined by observing that twitch contraction of constant amplitude could be initiated with this interval; the increase in the intensity or the duration caused further increase in the amplitude of the twitch contraction which, however, deteriorated gradually. This experiment was performed in the presence of d-tubocurarine (1 μM), so that the involvement of motor nerves including the endplate (Gruber, 1968) could be excluded. The extensor digitorum longus preparations were stimulated electrically in the same manner but using different stimulation parameters (500 μs duration, 20 V, 0.1 Hz).

The contraction of the preparations was measured isometrically by a force-displacement transducer (UL-10GR, Minebea, Nagano, Japan) and recorded with a thermal-pen recorder (RECTI-HORIZ-8K, NEC, Tokyo, Japan).

The rates of contraction (+dT/dt) and relaxation (−dT/dt) were measured simultaneously with the tension development of the twitch contraction by a differential amplifier (TYPE 1309, NEC San-ei, Tokyo). In this experiment, rates of contraction and relaxation divided by developed tension (+dT/dt/T and −dT/dt/T) were also measured.

Intracellular Ca^{2+} level measurement

The intracellular Ca^{2+} level ($[Ca^{2+}]_i$) was measured according to the method reported by Ozaki *et al.* (1987) with the fluorescent Ca^{2+} indicator fura-PE3. With fura-PE3, $[Ca^{2+}]_i$ can be measured without a significant decline of fluorescence for several hours (Kim *et al.*, 1995). The rat oesophageal striated muscle preparations (1 mm in width, 7 mm in length) were treated with the acetoxymethyl ester of fura-PE3 (fura-PE3/AM, 20 μM) for 5–6 h at room temperature. Pluronic F-127 (0.06%) was added to increase the solubility of fura-PE3/AM. After being loaded, the preparations were washed with modified Tyrode's solution at 37°C for 15 min to remove free fura-PE3/AM. Each preparation was held horizontally in a temperature-controlled organ bath (5 ml) connected by one end to a force-transducer. The preparations were illuminated by dual wavelength (340 and 380 nm) excitation light applied alternatively (128 Hz). The intensity of 500 nm fluorescence (F_{340} and F_{380}) was measured with a fluorometer (CAF-110, JASCO, Tokyo), and the ratio of F_{340} to F_{380} (F_{340}/F_{380}) was treated as an indicator of $[Ca^{2+}]_i$. The ratios obtained in Ca^{2+}-free Tyrode's solution containing 2 mM EGTA and in the presence of 142.4 mM K^+ were taken as 0 and 100%, respectively, and all other values were normalized using these two values.

Drugs

The drugs used in the present experiments were cyclopiazonic acid (CPA, Sigma, St. Louis, MO, U.S.A.), thapsigargin (Sigma), fura-PE3/AM (Dojindo, Kumamoto, Japan), pluronic F-127 (Sigma), ethylene glycol-*bis*(β-amino-ether)

N,N,N′,N′-tetraacetic acid (EGTA, Dojindo), d-tubocurarine (Sigma), 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulphonic acid (HEPES, Dojindo), 1-[β-[3-(4-Methoxy-phenyl)propoxyl]-4-methoxyphenethyl]-1H-imidazole hydrochloride (SK&F 96365, Biomol, PA, U.S.A.), flufenamic acid (Wako Chemicals, Osaka, Japan), mefenamic acid (Wako Chemicals) and verapamil hydrochloride (Wako Chemicals).

Statistics

The data obtained are expressed as means ± s.e.mean. The differences in the values obtained with preparations from WKY and SHRSP were analysed by Student's *t*-test. *P* values less than 0.05 were considered significant.

Results

Body weight and blood pressure of rats

The body weights of the SHRSP and WKY used in the present experiments were 265 ± 5.5 g ($n=28$) and 366 ± 4.8 g ($n=34$), respectively, the former being significantly smaller than the latter ($P<0.001$). The SBP values of the SHRSP and WKY were 256 ± 13.4 mmHg ($n=28$) and 137 ± 1.1 mmHg ($n=34$), respectively. The difference in the SBP was significant ($P<0.001$).

Effects of CPA on twitch contraction

Oesophageal striated muscle responded to the electrical stimulation showing twitch contraction. The height of the twitch contraction was decreased by the removal of extracellular Ca^{2+} but with much slower time course than the tonic response to CPA described below. CPA (10 μM) potentiated twitch contractions of the oesophageal striated muscle preparations from both WKY and SHRSP (Figure 1). Concentration-response experiment demonstrated that the threshold concentration for the potentiation of the twitch contraction was 0.3 μM and the maximal potentiation was observed at 10 μM in both preparations (Figure 1b). In the preparations from WKY, the maximal potentiation of twitch contraction (basal tension was subtracted) was observed 20 min after the application of CPA, and the amplitude of the twitch contractions at this point was 398 ± 33.1% ($n=11$) of that in the absence of CPA (Figure 1c). The SHRSP preparations showed the maximal twitch contraction 7 min after the application of CPA, and the amplitude was 204 ± 4.9% ($n=13$). The potentiation was significantly greater in the preparations from WKY ($P<0.001$) (Figure 1a, b and c). In the WKY preparations, an elevation of basal tension was observed, described below (Figure 1a).

The potentiation of the twitch contraction was associated with a change in the speed of tension changes (Figures 1 and 2). Although the maximal contraction speed was increased markedly by CPA (10 μM), especially in the preparations from WKY (Figure 2a), this effect was not significant when it was normalized by the tension of the twitch contraction (Figure 2c). The difference of the contraction speed between the preparations from WKY and SHRSP was minimized by this correction (Figure 2c). The relaxation speed, on the other hand, was reduced markedly (Figure 2b) and, after 20 min of the application of CPA, it was 46 ± 5.5 % ($n=9$) and 56 ± 3.3 % ($n=11$) of the control, respectively in the preparations from WKY and SHRSP. Unlike the rate of contraction, the effect of CPA on the rate

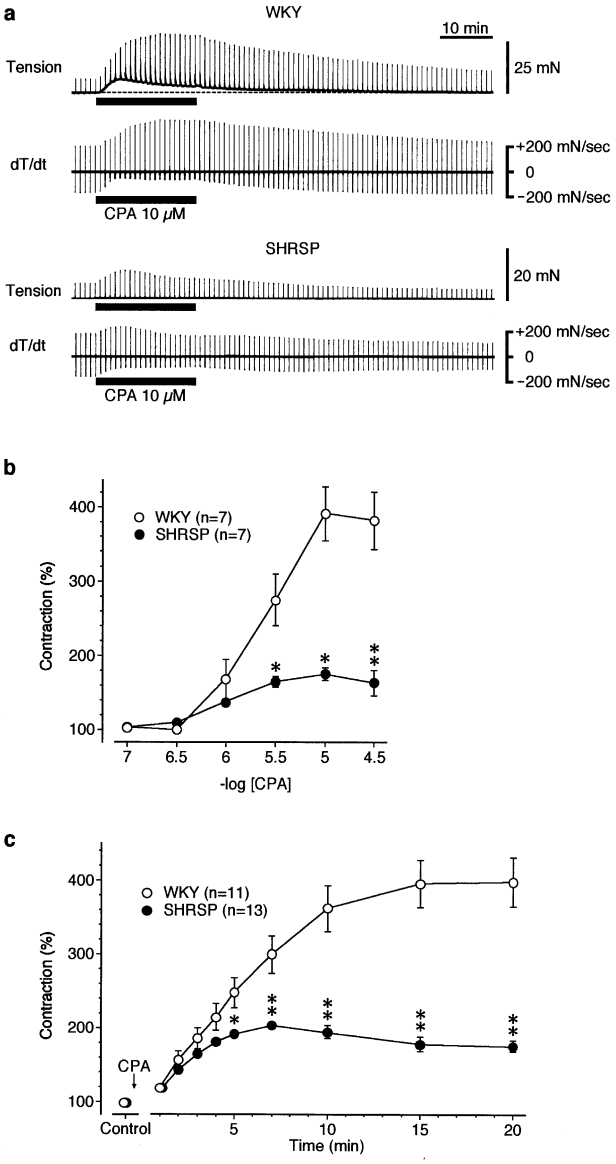

Figure 1 Effect of cyclopiazonic acid (CPA, 10 μM) on twitch contraction in the oesophageal striated muscle preparations from WKY and SHRSP. (a) Electrical stimulation (5 V, 10 ms duration) was applied every 60 s. dT/dt indicates the rate of contraction (+) or the rate of relaxation (−). (b) Concentration-response curves for CPA-induced potentiation of twitch contraction. Twitch contraction amplitude before the application of CPA was taken as 100% and the amplitude in the presence of CPA was expressed as percentages of this value. The elevation of the basal tension in the preparations from WKY was subtracted. (c) Time course of the effect of 10 μM CPA on twitch contraction in preparations from WKY and SHRSP. CPA was applied at the arrowhead. Data are expressed as the percentage of the amplitude of twitch contraction before CPA was added. Asterisks indicate significant differences from the data of preparations from WKY (*$P<0.05$, **$P<0.001$).

of relaxation was more pronounced when normalized with respect to twitch tension (Figure 2d).

Figure 3 shows the effect of CPA (10 μM) on the time course of the twitch contraction. As shown in Figure 3c, the time to 80% relaxation, especially that of the preparations from WKY, was markedly increased in the presence of CPA, while the time to peak tension was little affected in both preparations (Figure 3b).

It was also shown that CPA of the same concentration (10 μM) prolonged relaxing phase with decrease of relaxation speed in the skeletal muscle of the extensor digitorum longus

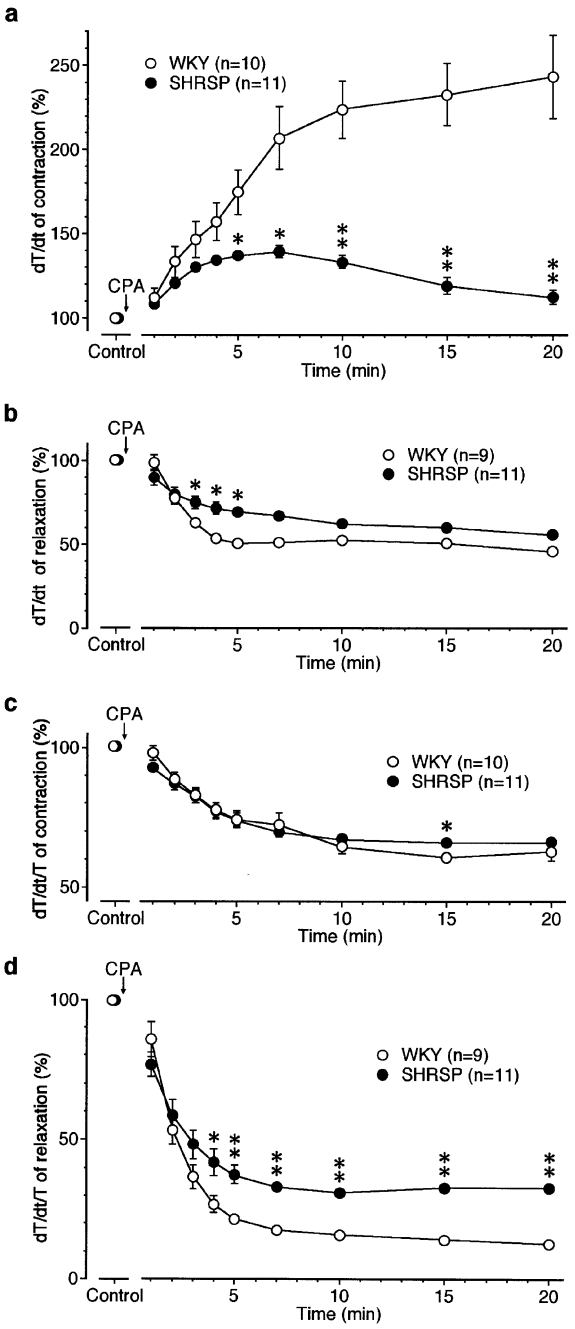

Figure 2 Time course of the effect of CPA on the speed of contraction and relaxation of twitch contraction. CPA of 10 μM was used in this experiment. Values were expressed as percentages of the values before the application of the drug. (a and b) Represent rates of contraction and relaxation (dT/dt), respectively. (c and d) Represent the rate of contraction and relaxation normalized by developed tension (dT/dt/T). CPA was applied at the arrowheads. Asterisks indicate significant differences from the data of preparations from WKY ($*P<0.05$, $**P<0.001$).

from both WKY and SHRSP. Potentiation of the twitch contraction and the elevation of the basal tension were not obvious in both preparations (data not shown).

Effects of CPA on the resting state

The effects of CPA on the tension and intracellular Ca^{2+} level ($[Ca^{2+}]_i$) were measured simultaneously in preparations

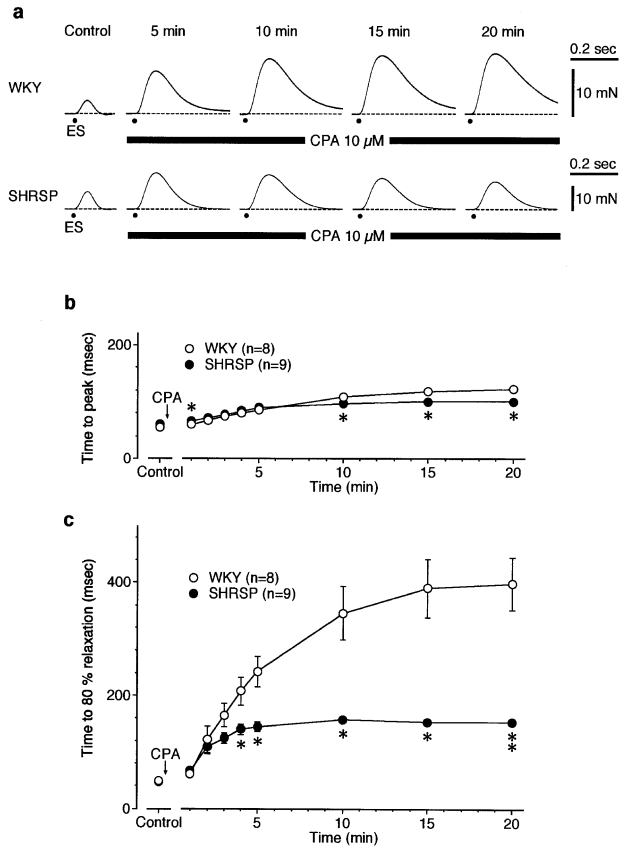

Figure 3 Effect of CPA (10 μM) on the duration of twitch contractions in the oesophageal striated muscle preparations from WKY and SHRSP. (a) Electrical stimulation (5 V, 10 ms duration) was applied every 60 s. Five to twenty min indicate the time from CPA application. The elevation of the basal tension in the preparations from WKY was subtracted. (b and c) Time course of the effect of CPA (10 μM) on time to peak (b) and time to 80% relaxation (c) of twitch contraction in preparations from WKY and SHRSP. CPA was applied at the arrowheads. Asterisks indicate significant differences from the data of preparations from WKY ($*P<0.05$, $**P<0.001$).

under the resting state. As in the stimulated preparations shown in Figure 1, the WKY preparations responded to CPA at a concentration of 10 μM showing a slow tension development, while preparations from SHRSP showed no or only a slight elevation of tension (Figure 4). The simultaneous observation of $[Ca^{2+}]_i$ showed that CPA of 10 μM caused an elevation of the level in both preparations (Figure 4). The effects of CPA on the resting state of these preparations are summarized in Figure 5. The differences in the tension development and the elevation of $[Ca^{2+}]_i$ between preparations from WKY and SHRSP were significant, being smaller in the preparations from SHRSP.

The CPA-induced tension development in the WKY preparations and the elevation of $[Ca^{2+}]_i$ in both preparations were sensitive to extracellular Ca^{2+}. In the absence of extracellular Ca^{2+}, CPA failed to induce the elevation of tension, although a small transient elevation of $[Ca^{2+}]_i$ was still observed (Figure 6). Similarly, both the developed tension and the elevated $[Ca^{2+}]_i$ in the presence of CPA disappeared when the extracellular Ca^{2+} was removed (Figure 7a). It should be noted that verapamil (10 μM) failed to exert any effect on either tension development or the elevation of $[Ca^{2+}]_i$ induced by CPA (Figure 7b).

Effects of nonselective cation channel inhibiting agents

This experiment was performed with the preparations from WKY, since both elevation of $[Ca^{2+}]_i$ and tension by CPA were observed in this preparation. The increase in $[Ca^{2+}]_i$ and contraction observed in the presence of CPA (10 μM) were depressed by flufenamic acid (100 μM) or by mefenamic acid (100 μM) (Figure 8a,b). Similarly, La^{3+} (100 μM) or Gd^{3+} (100 μM) reduced both the elevated $[Ca^{2+}]_i$ and the developed tension by CPA (data not shown). It was also shown that SK&F 96365 of 100 μM depressed both the elevated $[Ca^{2+}]_i$ and tension (Figure 8c), although the drug of the concentration of 10 μM did not.

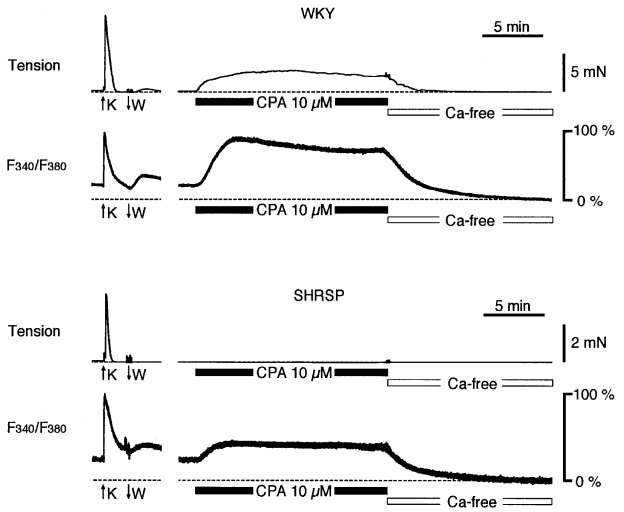

Figure 4 Effect of CPA (10 μM) on resting state of tension and $[Ca^{2+}]_i$ (F_{340}/F_{380}) in the oesophageal striated muscle preparations from WKY and SHRSP. K, W, CPA 10 μM and Ca-free indicate the application of 142.4 mM K^+ Tyrode's solution, washing out with modified Tyrode's solution, the application of 10 μM CPA and changing the solution to Ca^{2+}-free Tyrode's solution containing 2 mM EGTA, respectively.

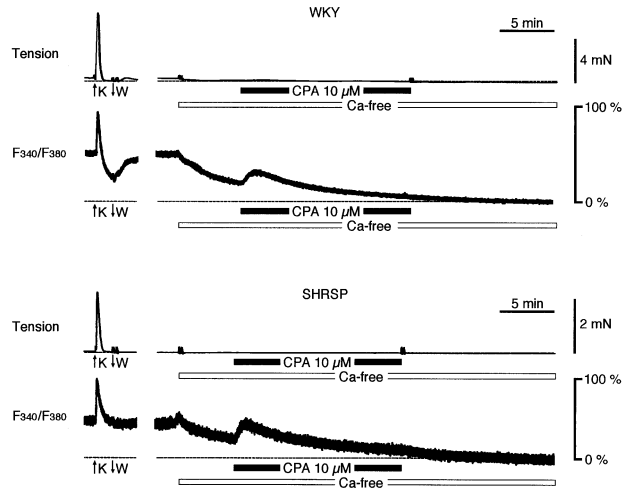

Figure 6 Effect of CPA on tension and $[Ca^{2+}]_i$ (F_{340}/F_{380}) in the absence of extracellular Ca^{2+} (Ca-free). CPA (10 μM) was added 5 min after the removal of extracellular Ca^{2+}. Other points are the same as those in Figure 4. CPA induced a transient increase in the intracellular Ca^{2+} level without tension development.

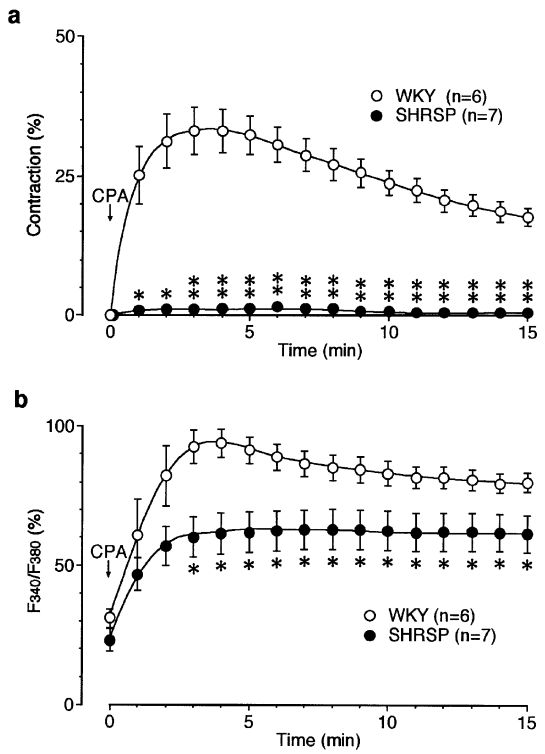

Figure 5 Time course of the developed tension (a) and the elevation of $[Ca^{2+}]_i$ (F_{340}/F_{380}, b) induced by CPA (10 μM) in preparations from WKY and SHRSP. CPA was added at the arrowheads. Data is expressed as a percentage of the developed tension (a) and the elevation of $[Ca^{2+}]_i$ (b) induced by 142.4 mM K^+ Tyrode's solution. Asterisks indicate significant differences from the data of the WKY preparations ($*P<0.05$, $**P<0.001$).

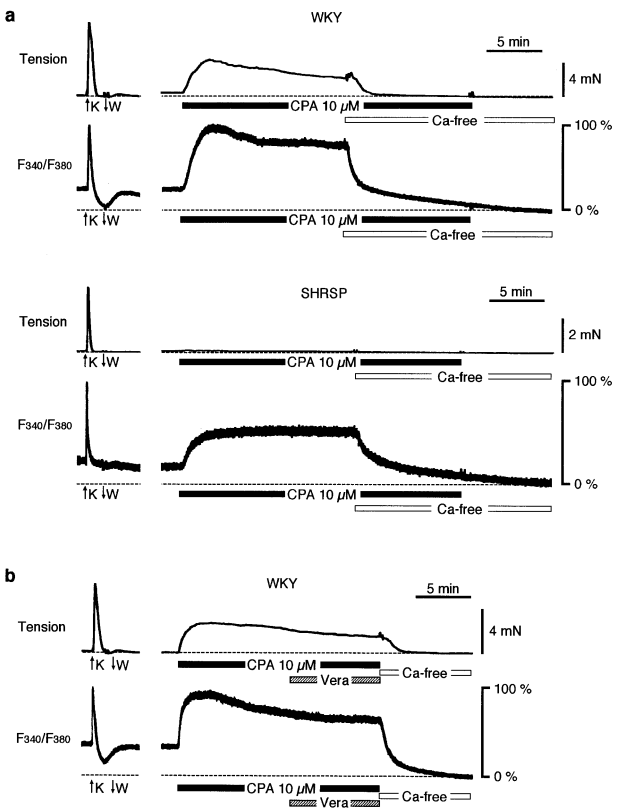

Figure 7 Effect of the removal of extracellular Ca^{2+} (a) and verapamil (b) on the tension and the $[Ca^{2+}]_i$ (F_{340}/F_{380}) elevated in the presence of CPA. The concentration of verapamil (Vera) was 10 μM. Other points are the same as those in Figure 4.

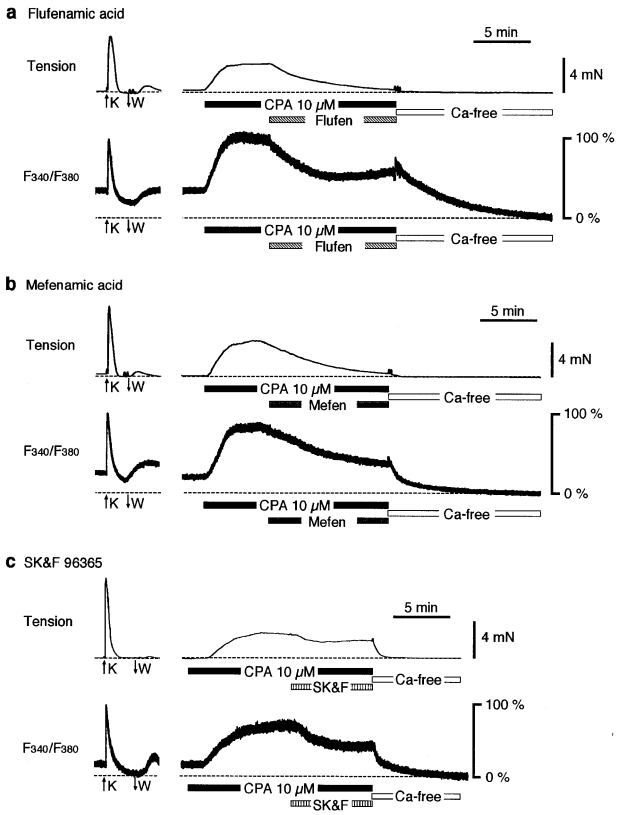

Figure 8 Effects of flufenamic acid (a), mefenamic acid (b) and SK&F 96365 (c) on the tension and $[Ca^{2+}]_i$ elevated in the presence of CPA. The concentrations of CPA, flufenamic acid (Flufen), mefenamic acid (Mefen) and SK&F 96365 (SK&F) were 10, 100, 100 and 100 μM, respectively. Other points are the same as those in Figure 4.

Effects of thapsigargin

Thapsigargin, at a concentration of 3 μM, produced an elevation of basal tension to the similar amplitude to that observed with 10 μM CPA in the preparations from WKY. The elevation was not observed in the preparations from SHRSP. The twitch contraction was not potentiated in both preparations as that observed with CPA, but rather depressed to $72.5 \pm 2.70\%$ ($n=6$) and $89.3 \pm 8.82\%$ ($n=6$), respectively in the preparations from WKY and SHRSP after 2 h the application. The speed of the relaxation and duration of the twitch contraction was markedly prolonged, although it could not be measured because of the appearance of small contraction on the relaxing phase (data not shown).

Discussion

It was demonstrated in the present experiments that the twitch contraction induced by electrical stimulation was markedly potentiated by CPA. This potentiation may be explained by (1) direct action of the drug on contractile proteins including sensitivity to Ca^{2+}, (2) changes in the Ca^{2+} level during contraction due to changes in Ca^{2+} handling, or (3) other factors.

It has been reported that the Ca^{2+} sensitivity of contractile proteins of slow skeletal muscle is increased by CPA (Huchet & Léoty, 1994). However, a high concentration of the drug was required to exhibit the effect. In addition, CPA of concentrations lower than 10 μM showed no effect on the pCa-tension relationship of contractile proteins of fast and slow skeletal muscle (Huchet & Léoty, 1994), cardiac muscle (Takahashi *et al*., 1995) and smooth muscle (Uyama *et al*., 1992). Thus, it is unlikely that the potentiation of twitch contraction is brought about by the direct action of CPA on contractile proteins.

The twitch contraction of oesophageal striated muscle is thought to be initiated by Ca^{2+} released from SR as in the skeletal muscle, since it persisted after the removal of extracellular Ca^{2+} with much slower time course than the sustained contraction by CPA. In the present study, the potentiation of twitch contraction induced by CPA was associated with a prolongation of the duration of contraction, in particular a prolongation of the time for relaxation caused by a slowed relaxation speed. This suggests that the potentiation of twitch contraction is due mainly to the slowing down of the relaxing activity. The slowing down of the relaxation can be explained by inhibiting action of CPA on SR Ca^{2+}-ATPase and Ca^{2+} uptake by SR (Goeger & Riley, 1989; Kurebayashi & Ogawa, 1991; Seidler *et al*., 1989). CPA of these concentrations (lower than 30 μM) would not reduce SR Ca^{2+} greatly, because of small amount of Ca^{2+} release during twitch contraction and enough time to replenish with the interval of the stimulation applied in the present experiment. Similar results have been reported in frog fast-twitch skeletal muscle (Même *et al*., 1998). It was shown that CPA potentiates the twitch contraction of skeletal muscle (extensor digitorum longus) prolonging the relaxation phase, although the potentiation was much less compared with that of oesophageal striated muscle from WKY. Since twitch contraction of skeletal muscle is initiated by released Ca^{2+} from SR (Somlyo *et al*., 1981; see Endo, 1977), this also supports the assumption that the potentiation of the twitch contraction of oesophageal striated muscle is due to the slowdown of Ca^{2+} accumulation by SR. The difference observed here in the action of CPA between preparations from WKY and SHRSP may thus be due to a difference in the properties of the SR, as has been reported in vascular smooth muscles (Dohi *et al*., 1990; Kojima *et al*., 1991; Toyoda *et al*., 1995; Nomura *et al*., 1997). The difference in the action of CPA has been reported between fast and slow cremaster striated muscles, and explained by the difference in the activity of SR (Huchet & Léoty, 1994).

However, it was shown in the present experiment that the relaxation time of twitch contraction in the absence of CPA was identical in the preparations from WKY and SHRSP. Then, it can be assumed that the relaxing activity of SR, Ca^{2+} pump activity, would be identical in both preparations. The sensitivity to CPA was identical, regardless of marked difference of the potentiating effect of twitch contraction (Figure 1b). Therefore, mechanism other than the difference of sensitivity to CPA may be involved in this difference. The possibility that the elevation of the basal Ca^{2+} concentration, described below, is a cause of potentiation, can not be entirely excluded, although it is known that the elevation of Ca^{2+} concentration usually accelerates the decline of $[Ca^{2+}]_i$ (Westerblad & Allen, 1993).

In the resting state of preparations from WKY, CPA induced tension development which was associated with an increase in $[Ca^{2+}]_i$. These changes were sensitive to extracellular Ca^{2+}, indicating the involvement of an influx of extracellular Ca^{2+} in the elevation of $[Ca^{2+}]_i$. In smooth muscle of the rat carotid artery, we found that both the contraction and the elevation of $[Ca^{2+}]_i$ induced by CPA were dependent on extracellular Ca^{2+} (Sekiguchi *et al*., 1996). It has also been reported that CPA increased the Ca^{2+} inward current of the smooth muscle of rat ileum (Ohta *et al*., 1995) and mouse anococcygeus (Wayman *et*

al., 1996), suggesting the involvement of Ca^{2+} influx in the contraction and elevation of $[Ca^{2+}]_i$ induced by CPA. These authors have suggested that the depletion of Ca^{2+} from the SR by CPA is the cause of the increase in the inward Ca^{2+} current. Such an effect of CPA has also been reported in cultured skeletal myoball (Hopf *et al*., 1996). The small but obvious increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} observed in the present experiment indicates the release which leads to the depletion of SR Ca^{2+} by CPA. The insensitivity of the contraction and the elevation of $[Ca^{2+}]_i$ induced by CPA to an L-type Ca^{2+} channel blocker observed in this experiment agrees with those reported in skeletal muscle (Hopf *et al*., 1996) and smooth muscle (Ohta *et al*., 1995; Wayman *et al*., 1996). Thus, the possibility that the increase in $[Ca^{2+}]_i$ and tension induced by CPA is brought about by the activation of Ca^{2+} release-activated Ca^{2+} channels (Parekh & Penner, 1997) is suggested. In this preparation, however, CPA would not deplete SR Ca^{2+} but reduce it, because twitch contraction did not disappear. Regarding to this, Oike & Ito (1997) reported that the Ca^{2+} release-activated Ca^{2+} entry mechanism is activated abruptly by a small amount of Ca^{2+} reduction in intracellular Ca^{2+} store site in bovine aortic endothelial cell. The result that thapsigargin, which is known to decrease Ca^{2+} uptake of SR (Sagara & Inesi, 1991; Wrzosek *et al*., 1992), induced the elevation of tension (contraction) in this preparation supports the possibility of the involvement of SR Ca^{2+}-reduction in the contraction.

It is difficult, however, to determine the type of the nonselective cation channels, since agents which block these channels, including Ca^{2+} release-activated Ca^{2+} channels, have been reported to be varied among the preparations (Hesheler & Schultz, 1993; Li & Van Breemen; 1996, Wiemann *et al*., 1998). In addition, no report is available as to the rat oesophageal striated muscle. In the present experiments, it was shown that several agents which have been reported to block nonselective inward currents, including Ca^{2+} release-activated nonselective cation currents, depressed the increase both in $[Ca^{2+}]_i$ and tension induced by CPA. Therefore, it can be concluded that both responses are, at least, initiated by increased Ca^{2+} influx through nonselective cation channels as reported in endothelial cells (Zhang *et al*., 1994). The possibility of the initiation of contraction by Ca^{2+} entered through nonselective cation channels has also been reported in endothelin-1-induced contraction of rabbit aorta (Komuro *et al*., 1997), although the mechanism of the opening of these channels may be different. It is also possible that the elevation of the basal tension is initiated by the elevated $[Ca^{2+}]_i$ due to an inhibition of extrusion of Ca^{2+} entered from extracellular space. However, this is thought to be less probable, since it has been reported that the Ca^{2+}-ATPase of skeletal muscle membrane was not inhibited by CPA (Seidler *et al*., 1989).

In the preparations from SHRSP, CPA did not or only slightly induce the elevation of the basal tension, although the drug increased $[Ca^{2+}]_i$. This may be explained by difference in the sensitivity of contractile apparatus to Ca^{2+}. However, no study has been done on the difference in the sensitivity of contractile protein to Ca^{2+} in between striated muscles of WKY and SHRSP. Since the elevation of the basal tension was less than 35% of the amplitude of high K^+ contraction in the preparations from WKY and since F_{340}/F_{380} expresses logarithm of Ca^{2+}, the difference in absolute value of $[Ca^{2+}]_i$, which was lower in the preparations from SHRSP as compared with those from WKY, may also explain the difference in the contraction. A reduction in the increase in $[Ca^{2+}]_i$ produced by CPA in SHRSP preparations as opposed to WKY preparations may be due to a decrease in the susceptibility of the SR to CPA. This possibility was supported by the reduced effect of CPA on the twitch contraction in SHRSP preparations.

In skeletal muscle, it has been established that the elevation of $[Ca^{2+}]_i$ and the associated contraction in E-C coupling is initiated by the release of Ca^{2+} from the SR (Endo, 1977). The net Ca^{2+} release from the SR due to normal Ca^{2+} leakage and inhibited Ca^{2+} uptake into the SR would be an alternative explanation for the increase in $[Ca^{2+}]_i$ and the contraction induced by CPA. It has been observed that caffeine-induced contraction (which is thought to be initiated by the release of Ca^{2+} from the SR) of oesophageal striated muscle was gradually reduced by the removal of extracellular Ca^{2+} (Sekiguchi *et al*., unpublished observation), indicating some interdependency between SR Ca^{2+} and extracellular Ca^{2+}. However, this explanation of the action of CPA by the release of SR Ca^{2+} is less probable, since the time course of the decay of caffeine-induced contraction by the removal of extracellular Ca^{2+} was much slower than that of the CPA-induced contraction.

In conclusion, in the rat oesophageal striated muscle, CPA potentiated twitch contractions by inhibiting the Ca^{2+} sequestrating activity of the SR. The potentiation was more prominent in preparations from WKY. The difference in the action of CPA between WKY and SHRSP preparations may be due to a difference in the reactivity of the SR to CPA. In the resting state, CPA seems to activate Ca^{2+} release-activated Ca^{2+} channels and to elevate $[Ca^{2+}]_i$. The elevation of $[Ca^{2+}]_i$ was greater in the preparations from WKY and the resting tension was also elevated, while the elevation of $[Ca^{2+}]_i$ was smaller and no tension development was observed in the preparations from SHRSP. The difference may be explained by a difference in the reactivity of the SR to CPA or by the difference in channels for the Ca^{2+} release-activated Ca^{2+} currents. The cause of the difference in the reactivity to CPA remained to be further investigated.

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