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Effects of cyclopiazonic acid and thapsigargin on electromechanical activities and intracellular Ca^{2+} in smooth muscle of carotid artery of hypertensive rats

[1]F. Sekiguchi, *K. Shimamura, M. Akashi & S. Sunano

Faculty of Pharmaceutical Sciences, Kinki University, Kowakae, Higashi-Osaka, Japan and *Research Institute of Hypertension, Kinki University, Osaka-Sayama, Japan

1 The effects of cyclopiazonic acid (CPA) and thapsigargin (TG), both of which are known to inhibit sarcoplasmic reticular Ca^{2+}-ATPase, on the mechanical activities, intracellular Ca^{2+} level and electrical activities of smooth muscle of the carotid artery of stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar Kyoto rats (WKY) were compared.

2 Both CPA and TG induced elevation of tension of the smooth muscle, which was composed of a phasic and a tonic component. The level of tension attained, especially the tonic component, was greater in the preparation from SHRSP.

3 The elevation of tension was associated with an increased intracellular Ca^{2+} level. Both the elevation of tension and the increase in intracellular Ca^{2+} were diminished by the removal of extracellular Ca^{2+} or by the application of verapamil.

4 The resting membrane potential of the preparations from SHRSP were depolarized to a greater extent than those from WKY. CPA depolarized the smooth muscle from both SHRSP and WKY, and the final level was also more depolarized in the preparation from SHRSP.

5 These results indicate that the elevation of tension induced by these drugs is mainly due to increased Ca^{2+} influx through voltage-dependent Ca^{2+} channels, and the difference in the action between the preparation from SHRSP and that from WKY can be explained mainly by the changes in the channels.

6 Thus, differences in the action of these drugs on the tension of smooth muscle between preparations from WKY and SHRSP can mainly be explained by the difference in the membrane potential which is related to the difference in voltage-dependent Ca^{2+} influx.

Keywords: Stroke-prone spontaneously hypertensive rat; carotid artery; smooth muscle; cyclopiazonic acid; thapsigargin; contraction; intracellular Ca^{2+}; membrane potential

Introduction

Cyclopiazonic acid (CPA) and thapsigargin (TG), inhibitors of endoplasmic reticulum Ca^{2+}-ATPase (Thastrup *et al.*, 1990; Missiaen *et al.*, 1992), have been shown to inhibit Ca^{2+} uptake and to deplete Ca^{2+} content of the sarcoplasmic reticulum in skeletal and heart muscle (Seidler *et al.*, 1988; Goeger & Riley, 1989; Janczewski & Lakatta, 1993). These drugs are therefore used as tools to study excitation-contraction coupling in these muscles. In some smooth muscles, these drugs have been shown to cause contraction (Uyama *et al.*, 1993; Low *et al.*, 1993), and they were also demonstrated to elevate the intracellular Ca^{2+} concentration (Sill *et al.*, 1991; Baro & Eisner, 1992; Xuan *et al.*, 1992; Alexander & Cheung, 1994). However, the contraction and increase in intracellular Ca^{2+} induced by these drugs in most smooth muscles are sensitive to extracellular Ca^{2+} and cannot be explained simply by their action on the sarcoplasmic reticulum (Xuan *et al.*, 1992; Mikkelsen *et al.*, 1988; Low *et al.*, 1991; Uyama *et al.*, 1993), although Baro & Eisner (1992) reported that the action of TG could be explained simply by an action on the sarcoplasmic reticulum.

Xuan *et al.* (1992) reported that TG stimulates both nicardipine-sensitive and -insensitive Ca^{2+} entry. In addition, the effects of these drugs on electrical activities of smooth muscle cell membrane have also been determined (Suzuki *et al.*, 1992; Uyama *et al.*, 1992; Maggi *et al.*, 1995). Thus, the actions of CPA and TG on smooth muscle are rather complicated.

In the vascular smooth muscle of hypertensive rats, membrane excitation, Ca^{2+} entry and Ca^{2+} handling by the sarcoplasmic reticulum are known to be altered (Kwan, 1985; Cauvin *et al.*, 1989; Cheung, 1989). Therefore, it is of interest to compare the effects of these drugs on the blood vessels of normotensive and hypertensive rats. In the present study, the effects of CPA and TG on smooth muscles of carotid arteries from stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive Wistar Kyoto rats (WKY) were compared. Since it has been shown that these drugs also act on the endothelium and the factors released from the endothelium influence the contraction of smooth muscle (Mikkelsen *et al.*, 1988; Macarthur *et al.*, 1993; Moritoki *et al.*, 1994), all of the experiments were performed with endothelium-denuded preparations.

Methods

SHRSP and WKY were obtained from Dr Okamoto and successively bred and fed 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. Blood pressure was measured by the tail cuff method after the animals were warmed at 40°C for 10 min.

The rats were killed at 16 weeks of age by exsanguination from the vena cava under ethyl ether anesthesia. The carotid arteries were then removed and immersed in a modified Tyrode 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. High-K^+ solution was made by replacing NaCl in the modified Tyrode solution with equimolar KCl. Ca^{2+}-free

[1]Author for correspondence.

Tyrode solution was made by omitting $CaCl_2$ with or without adding 2.0 mM ethylene glycol-*bis* (β-amino-ether)*N,N,N',N'*-tetraacetic acid (EGTA).

For the contraction experiments, spiral preparations 1 mm in width and 15 mm in length were made from the common carotid artery. Endothelium was removed by rubbing the inner surface with cotton. One end of the preparation was connected with a silk thread to the apparatus for tension recording filled with the modified Tyrode solution (37°C) and the other was connected to a force-displacement transducer with a tungsten wire. The preparations were stretched to 150% of relaxed length, and equilibrated in the modified Tyrode solution for at least 60 min. Then, the preparations were subjected to high-K^+-induced contraction twice for 20 min each time with an interval of 30 min by changing the solution to high-K^+Tyrode solution containing 80 mM K^+. The experiments with CPA or TG were performed after these procedures. Since the effects of CPA and TG were only slightly reversible, only one contraction by these drugs was obtained in each preparation. At the end of mechanical experiments, the preparations were relaxed completely by applying verapamil (10^{-5} M) and papaverine (10^{-4} M) and tensions were measured from this relaxed level.

Changes in intracellular Ca^{2+} concentrations were determined by the fura-2 method (Grynkiewicz *et al.*, 1985). Endothelium-denuded spiral preparations 1 mm in width and 10 mm in length were loaded with fura-2/AM by incubation in Tyrode solution containing 20 μM fura-2/AM and 0.08% cremophor EL for 4 h at 18°C. The preparation was then mounted on the intracellular ion measurement apparatus (Nihon-Bunkoh, CAF-110) and intracellular Ca^{2+} concentrations were measured as the ratio of the luminescence excitation at 340 nm and 380 nm wave length. The minimum level of intracellular Ca^{2+} was determined by soaking the preparations in Ca^{2+}-free Tyrode solution containing 2 mM EGTA. The changes in Ca^{2+} concentrations were expressed as percentages of tonic phase of the elevation induced by high-K^+ Tyrode solution. One end of the preparation was connected to a force-displacement transducer so that the changes in tension could be measured simultaneously.

The membrane potential was measured by the microelectrode technique. Arterial rings from WKY and SHRSP were cut open and endothelium was removed by rubbing the intimal surface with cotton. They were mounted on a silicon rubber bed chamber (volume 1 ml) which was continuously perfused with warm modified Tyrode solution at a rate of 4 ml min^{-1}. Preparations were stretched to 150% of their relaxed length in a circumferential direction and pinned on the bed. A glass capillary microelectrode filled with 3 M KCl and with a tip resistance of 40 to 60 MΩ was impaled from the intimal surface, and signals obtained through a microelectrode amplifier (Nihon Kohden, MEZ 8201) were recorded on an ink pen recorder (NEC san-ei, 8561 ME-1H) and a data tape recorder (Nihon Kohden, RMG 5304). Intracellular recording was accepted as valid when an abrupt change in recorded voltage were observed at impalement and dislodgement.

Drugs used in this experiment were cyclopiazonic acid (CPA, Sigma, St. Louis, U.S.A.), thapsigargin (TG, Sigma, St. Louis, U.S.A.), fura-2/AM (Dojindo, Japan), ethylene glycol-*bis* (β-amino-ether)*N,N,N',N'*-tetraacetic acid (EGTA, Dojindo, Japan), verapamil hydrochloride (Wako Chem., Japan), cremophor EL (Nakarai Tesque., Japan) and caffeine (Wako Chem., Japan).

Obtained values are expressed as means ± s.e. The differences in the values were analyzed by Student's *t* test or paired *t* test. *P* values less than 0.05 were considered significant.

Results

Body weight and blood pressure of the rats

Body weight of SHRSP and WKY was 308.0 ± 4.8 g and 386.0 ± 6.8 g, respectively, the former being significantly smaller ($P<0.001$). Systolic blood pressure was 249.1 ± 3.5 mmHg and 133.6 ± 1.5 mmHg in SHRSP and WKY, respectively, and the difference between the two was significant ($P<0.001$).

CPA- or TG-induced contraction in SHRSP and WKY carotid arteries

The preparation from SHRSP showed spontaneous elevation of tension (tone) relative to the level observed in the presence of verapamil and papaverine, while no such elevation of tension was observed in the preparation from WKY. CPA (10^{-5} M) induced elevation of tension in preparations from both SHRSP and WKY (Figures 1 and 2a). The increase in tension of the preparation from WKY was composed of a phasic and a small tonic component. The maximum amplitude of the increase in tension was much smaller than that induced by 80 mM K^+. In the preparation from SHRSP, the increase in tension was composed of a phasic component of similar amplitude to that observed in the preparation from WKY and a large tonic component. Tension oscillations were often seen in the course of the tonic component (Figure 1). The amplitude of the maximum tension, expressed as percentage of the 80 mM K^+-induced contraction, was greater in the preparation from SHRSP than that from WKY and closer to that of the contraction induced by high-K^+.

TG at a concentration of 10^{-6} M induced increased tension with a similar amplitude. The increase was composed of two components, both of which were greater in the preparation from SHRSP than in that from WKY (Figure 2b).

Effects of Ca^{2+}-removal or Ca^{2+} antagonist on CPA- or TG-induced contraction

The tone of SHRSP carotid artery was abolished by Ca^{2+} removal or by the application of verapamil (10^{-5} M). The increase in tension induced by CPA was abolished by the re-

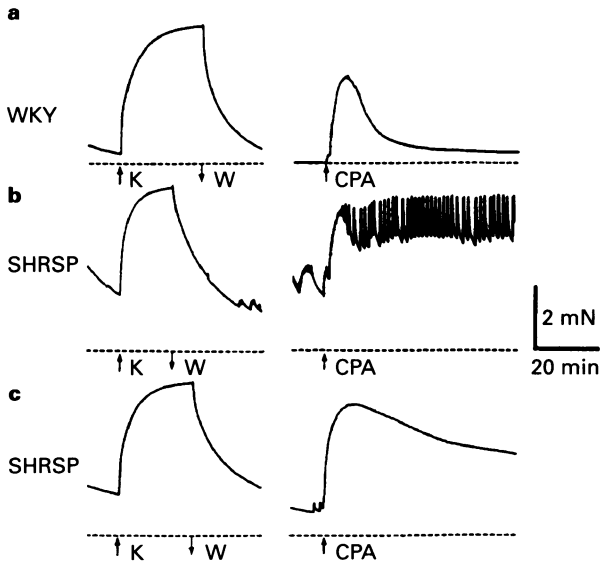

Figure 1 Effects of cyclopiazonic acid (CPA) on mechanical activities of endothelium-denuded carotid artery preparations from WKY and SHRSP. CPA-induced contraction from SHRSP with (b) and without tension oscillation (c) are shown. The increase in tension of the preparations from WKY did not show the tension oscillation (a). K, W and CPA indicate the application of high-K^+ Tyrode solution containing 80 mM K^+, washing out with normal Tyrode solution and the application of CPA (10^{-5} M), respectively. Dotted lines indicate the relaxed level in the presence of verapamil and papaverine (see Methods). Note the difference in the tonic component of the increase in tension.

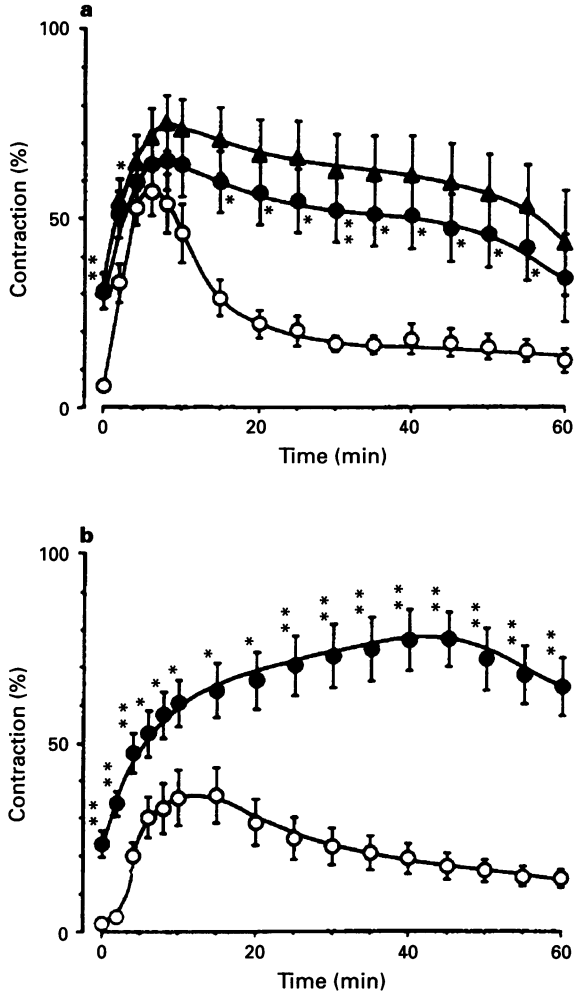

Figure 2 Time course of the increase in tension by cyclopiazonic acid (CPA) and thapsigargin (TG). (a) The increase in tension induced by CPA at a concentration of 10^{-5} M: (○), (▲) and (●) indicate time course of the mean elevation of tension of the preparation from WKY ($n=7$), the elevation of tension of the preparation from SHRSP ($n=16$) measured at the top of the tension oscillation and that measured at the foot of the tension oscillation, respectively. (b) The increase in tension induced by TG at a concentration of 10^{-6} M: (○) and (●) indicate the elevation of tension of the preparations from WKY ($n=9$) and SHRSP ($n=12$), respectively. Increase in tension is expressed as a percentage of that induced by high-K^+ Tyrode solution. The difference at time 0 was due to the difference in basal tone. Asterisks indicate significant differences from the increase in tension of the preparation from WKY (*$P<0.05$, **$P<0.001$). The maximum contractions of the preparations from SHRSP were significantly greater than those of the preparations from WKY ($P<0.001$). Note the difference in the tonic component of the contraction of the preparations from WKY and SHRSP.

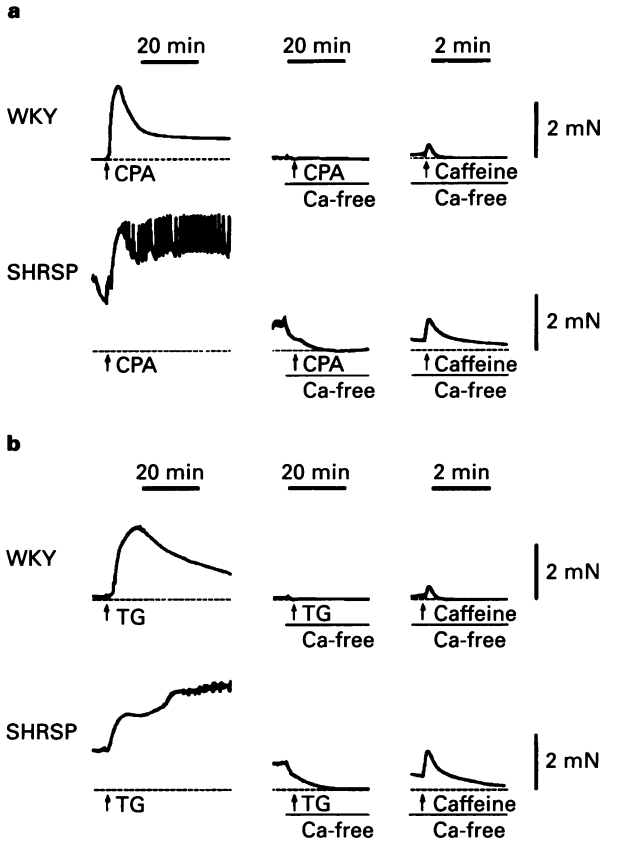

Figure 3 Effects of extracellular Ca^{2+}-removal on the increase in tension induced by CPA (a) and TG (b). CPA, TG and Caffeine indicate the application of CPA (10^{-5} M), TG (10^{-6} M) and caffeine (30 mM), respectively. These drugs were applied 3 min after the removal of extracellular Ca^{2+} (Ca-free). Each trace represents a result from different preparation (see Methods). Note the difference in the speed of the recording. Dotted lines are relaxed level as in Figure 1.

moval of extracellular Ca^{2+} in all of 5 and 4 preparations from WKY and SHRSP, respectively (Figure 3). In the preparation from SHRSP, both spontaneous and CPA-induced tension oscillations were also abolished in Ca^{2+}-free solution. Under the conditions where CPA-induced increase in tension was abolished, caffeine could still induce contraction, although the contraction was reduced by extracellular Ca^{2+} removal as compared with that in the presence of extracellular Ca^{2+} ($60.4\pm2.66\%$ ($n=8$) and $88.5\pm3.26\%$ ($n=10$) in the preparation from WKY and SHRSP, respectively).

The Ca^{2+} antagonist, verapamil (10^{-5} M) attenuated the elevation of tension induced by CPA in the preparation from WKY, but the phasic component was still observed (Figure 4a). The attenuation was much more prominent in the preparation from SHRSP and only a small tonic component was observed in the presence of verapamil. Both spontaneous tone and CPA-induced tension oscillations were also abolished by verapamil (Figure 4b). Similar effects to those of verapamil were obtained with nifedipine (10^{-6} M); the effects were more prominent in the preparation from SHRSP as observed with verapamil.

The effects of Ca^{2+}-removal and verapamil were observed on the elevation of tension induced by TG. The elevation was abolished in the absence of extracellular Ca^{2+} (Figure 3), and the application of verapamil (10^{-5} M) attenuated TG-induced elevation of tension (Figure 4c,d). The effect of verapamil was also more prominent in the preparation from SHRSP, although the remaining tonic component in the presence of verapamil was slightly greater in TG-induced elevation of tension when compared with that induced by CPA. Nifedipine (10^{-6} M) exhibited the same effects as those of verapamil on TG-induced elevation of tension.

Effects of CPA on intracellular Ca^{2+}

CPA caused an increase in intracellular free Ca^{2+} concentration composed of phasic and tonic phases in the preparations from both SHRSP and WKY. The increased level was slightly but significantly higher in the preparation from SHRSP. The intracellular Ca^{2+} concentration before the application of CPA was already slightly higher in the preparation from SHRSP than in that from WKY (Figure 5). In 4 of 18 SHRSP preparations tested, oscillation was observed during the tonic component of the increase in the intracellular Ca^{2+} level.

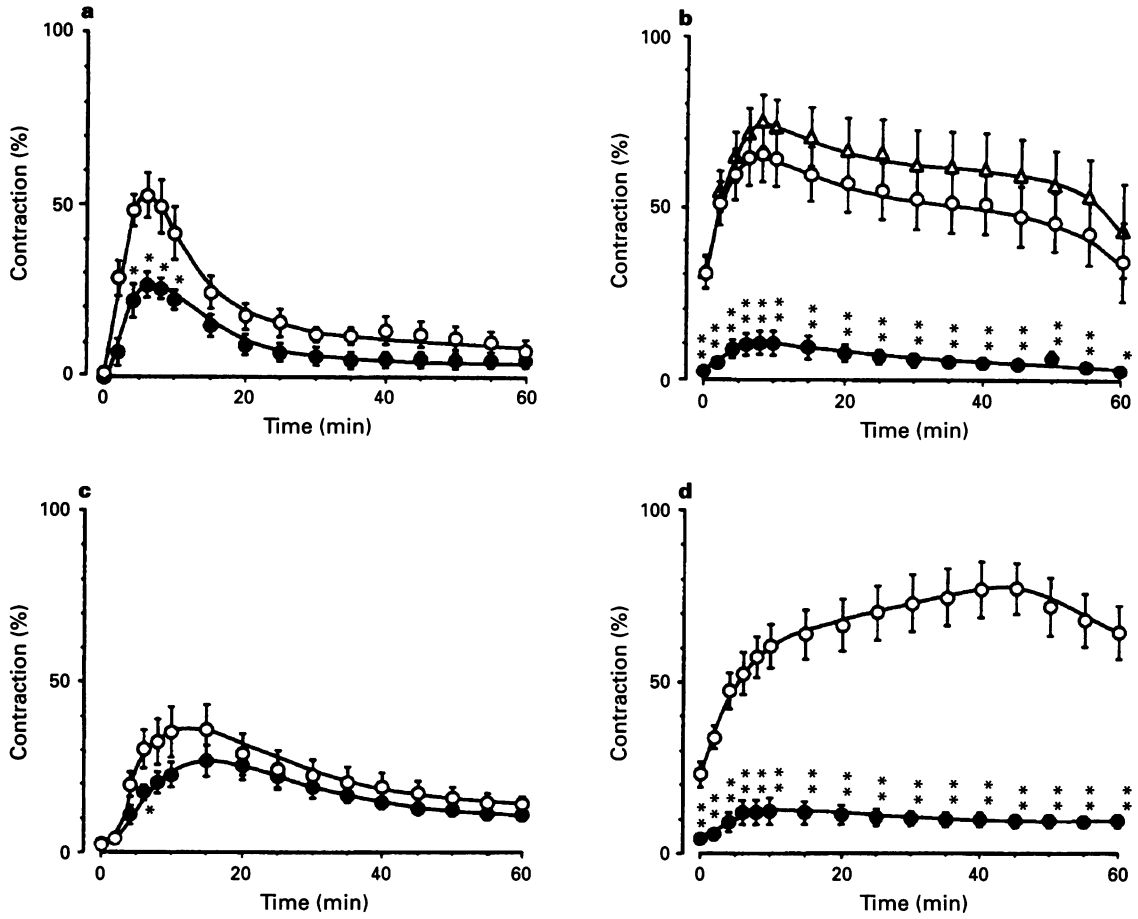

Figure 4 Effects of verapamil on the tension increase induced by CPA and TG. CPA (10^{-5} M) or TG (10^{-6} M) was applied in the absence and presence of verapamil (10^{-5} M). (○) and (△) indicate tension measured at the foot of tension oscillation and tension measured at the peak of tension oscillation, respectively. (●) indicates tension increase in the presence of verapamil. (a and b) Effect of verapamil on CPA-induced tension increase in the preparations from WKY and SHRSP, respectively. (c and d) Effect of verapamil on TG-induced tension increase in preparations from WKY and SHRSP, respectively.

The elevation of the intracellular Ca^{2+} level induced by CPA was greatly reduced in the absence of extracellular Ca^{2+} but a small phasic increase was still observed (Figure 6), although the simultaneous recording of mechanical response showed no increase in tension. The Ca^{2+} concentration in Ca^{2+}-free Tyrode solution was also higher in the preparation from SHRSP.

CPA induced elevation of intracellular Ca^{2+} level in the presence of verapamil (10^{-5} M), although the degree of this increase was markedly reduced compared to that in the absence of this agent. Simultaneous recording of tension demonstrated that the increase in Ca^{2+} concentration could initiate elevation of tension of reduced amplitude (Figure 7).

Effects of CPA on the membrane potential

The smooth muscle cells of the carotid artery of WKY were electrically quiescent, while a small amount of spontaneous activity was sometimes observed in the preparations from SHRSP. The resting potential of the smooth muscle cells of endothelium-denuded preparations from WKY was -51.0 ± 1.0 mV ($n=18$) and that of the smooth muscle cells from SHRSP was -32.1 ± 1.3 mV ($n=16$). Thus, the smooth muscle cells of SHRSP had significantly depolarized membrane potential ($P<0.05$).

CPA (10^{-5} M) induced depolarization of the cell membrane in endothelium-denuded preparations from both WKY and SHRSP. The membrane reached maximal depolarization around 5 to 7 min after the application (Figure 8). In the present experiments with the same preparation, it was shown that CPA depolarized the membrane from -52.2 ± 5.1 mV to -42.0 ± 4.8 mV ($n=5$) in WKY and from -35.3 ± 2.5 mV to -21.0 ± 2.8 mV ($n=4$) in SHRSP ($P<0.05$). The depolarized level of the membrane potential in the presence of the drug was significantly more positive in the preparation from SHRSP.

The smooth muscle of the carotid artery from SHRSP often exhibited spike-like or oscillatory potential changes, while no such activity was observed in the preparations from WKY. Accordingly, the first phase of the response to CPA was rather complicated showing spike-like and oscillatory activity (Figure 8).

Discussion

Physiological contraction of the smooth muscle is initiated by elevation of the intracellular Ca^{2+} concentration and the intracellular Ca^{2+} concentration is the determinant factor of the tension developed by contractile proteins. The elevation of intracellular Ca^{2+} concentration is thought to be brought about by influx of extracellular Ca^{2+} and release of Ca^{2+} from the sarcoplasmic reticulum. The influx of Ca^{2+} is achieved through voltage-sensitive and -insensitive pathways in the membrane (Bolton, 1979). In the vascular smooth muscle of spontaneously hypertensive rats, it has been reported that these mechanisms are altered toward the potentiation of contraction (Cauvin, 1986). In addition, the membrane electrical activities have also been reported to be altered (Cheung, 1989; Fujii *et al.*, 1993).

CPA and TG, which have been shown to inhibit sarcoplasmic reticular Ca^{2+}-ATPase in skeletal and caridac muscles, are used as a tool for the study of excitation-contraction

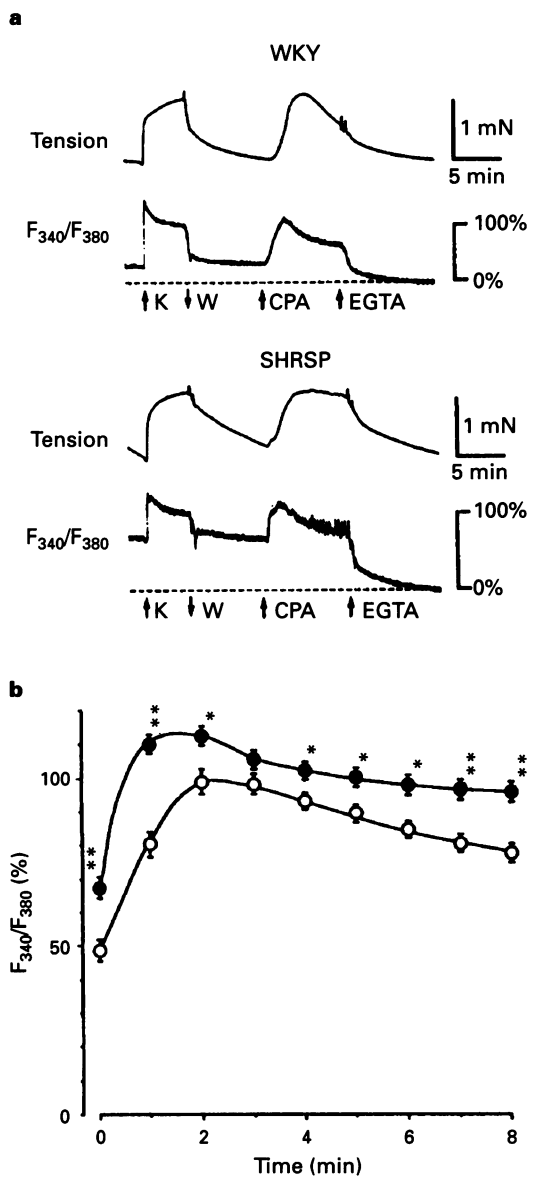

Figure 5 Effects of CPA on intracellular Ca^{2+} concentration of the smooth muscle of carotid arteries from WKY and SHRSP. (a) Simultaneous tracings of the tension (top) and intracellular Ca^{2+} level (bottom) of the preparation from WKY and SHRSP. Details are described in the Methods section. K, W, CPA and EGTA indicate application of high-K^+ Tyrode solution, washing out with normal Tyrode solution, the application of 10^{-5} M CPA and Ca^{2+}-free Tyrode solution containing 2 mM EGTA, respectively. Dotted lines indicate the Ca^{2+} concentration observed in the presence of EGTA. (b) Time course of the changes in intracellular Ca^{2+} level in the preparations from WKY (○, $n=15$) and SHRSP (●, $n=18$). Intracellular Ca^{2+} levels are expressed as percentages of the change in Ca^{2+} level induced by the application of high-K^+ Tyrode solution. Asterisks indicate significant differences from the values of the preparations from WKY.

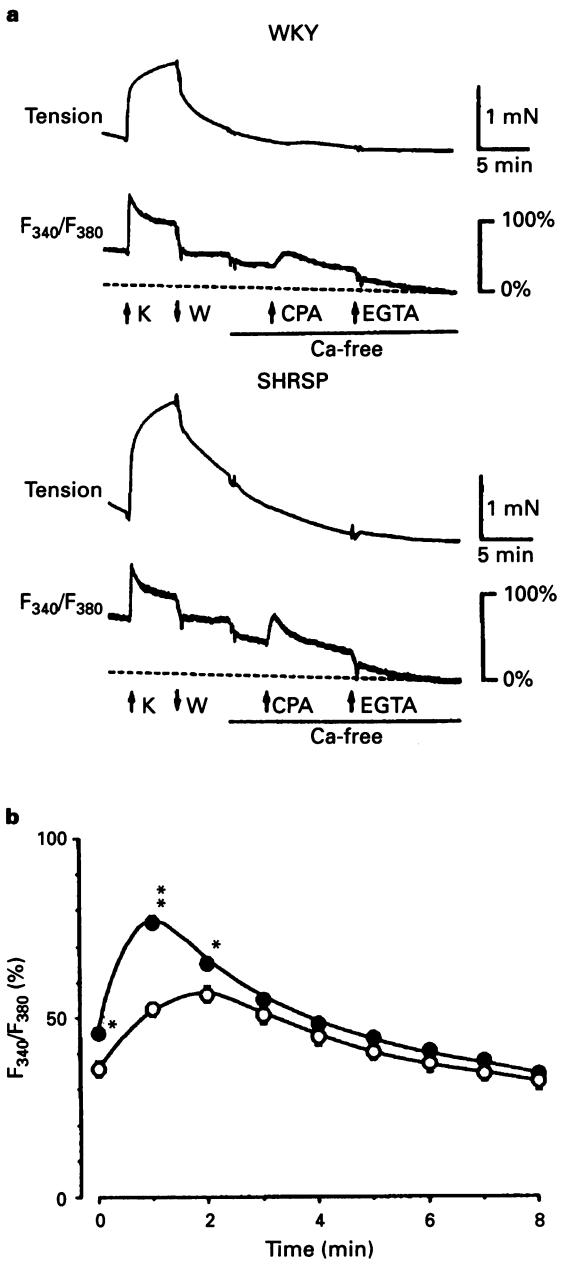

Figure 6 Effects of extracellular Ca^{2+} removal on the change in intracellular Ca^{2+} concentration induced by CPA. CPA (10^{-5} M) and EGTA (2 mM) were applied in the absence of extracellular Ca^{2+} (Ca-free). Other points are the same as those in Figure 5 (WKY: $n=9$, SHRSP: $n=9$).

coupling in these muscles (Janczewski & Lakatta, 1993; Lewartowski *et al.*, 1994; Chiesi *et al.*, 1994; Yard *et al.*, 1994). These drugs are also used in smooth muscle to deplete sarcoplasmic reticular Ca^{2+} or to inhibit Ca^{2+} uptake by the sarcoplasmic reticulum (Sill *et al.*, 1991; Low *et al.*, 1991; Uyama *et al.*, 1992; Low *et al.*, 1992; Luo *et al.*, 1993; Alexander & Cheung, 1994; Kwan *et al.*, 1994; Naganobu *et al.*, 1994). However, it is also known that these drugs cause an increase in the influx of extracellular Ca^{2+} (Xuan *et al.*, 1992). The possibility that these agents induce changes in membrane electrical activity has also been reported in the intestinal smooth muscle (Uyama *et al.*, 1993). In addition, their potential action on endothelium has also been reported in the blood vessels (Mikkelsen *et al.*, 1988; Macarthur *et al.*, 1993; Moritoki *et al.*, 1994). Thus, the multiple sites of action should be considered when the action of these drugs are discussed.

The present mechanical experiments were performed with endothelium-denuded preparations so that the effects on the smooth muscle alone could be observed. Using these preparations, it was demonstrated that the smooth muscle of the carotid artery exhibited an elevation of tension in response to the application of CPA or TG, and that the elevation of tension was reduced in the presence of verapamil and abolished by the removal of extracellular Ca^{2+}. The reduction of the increased tension in the presence of verapamil indicates the involvement of the voltage-dependent pathway of Ca^{2+} influx. The increased tension remaining in the presence of verapamil is

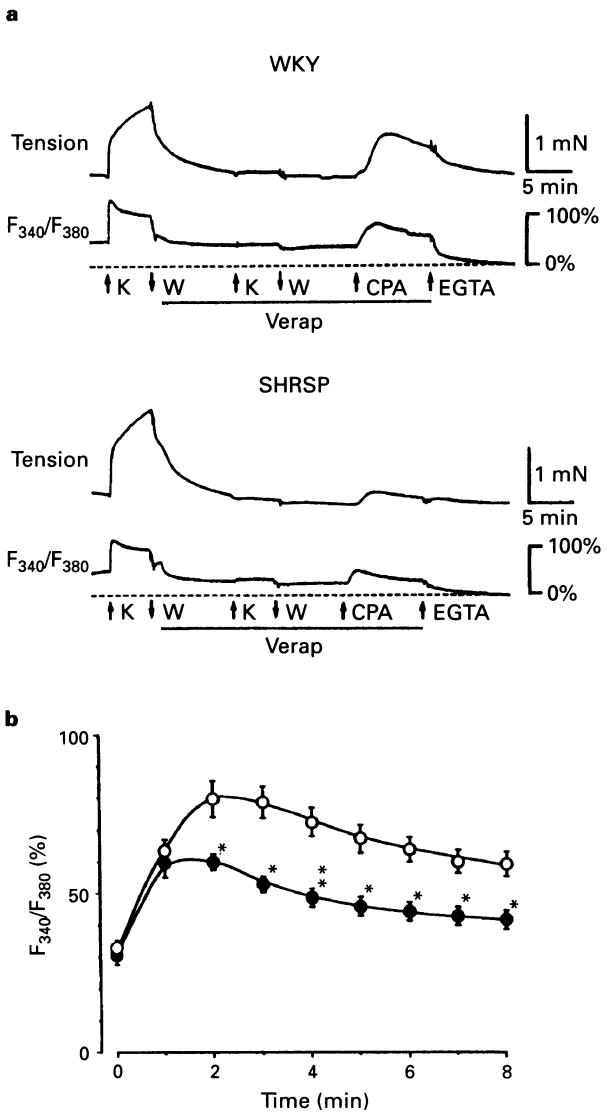

Figure 7 Effects of verapamil on the change in intracellular Ca^{2+} level induced by CPA. (a) Simultaneous recordings of changes in tension and intracellular Ca^{2+} level. CPA (10^{-5} M) was applied in the presence of 10^{-5} M verapamil (Verap) where high-K^+-induced tension development was abolished. (b) Time course of the changes in intracellular Ca^{2+} concentrations expressed as percentages of the change induced by high-K^+. Other points are the same as those in Figure 5. Compare the curves in Figure 5 (WKY: $n=7$, SHRSP: $n=7$).

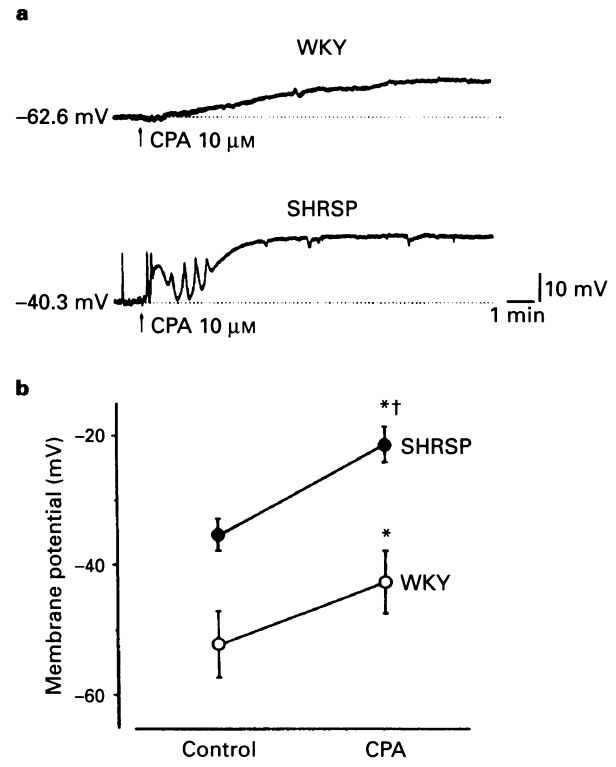

Figure 8 Effects of CPA on the membrane potential of smooth muscle of the carotid artery from WKY and SHRSP. (a) Typical traces of the change in membrane potential. CPA was used at 10^{-5} M. Note spontaneous or CPA-induced changes in the membrane potential in the prepartion from SHRSP. (b) Steady-state membrane potential before (Control) and after the application of 10^{-5} M CPA in the preparations from WKY (○, $n=7$) and SHRSP (●, $n=4$). Asterisks and daggers indicate significant differences between the membrane potential before and after application of the drug, and between the membrane potentials of SHRSP and WKY, respectively ($P<0.05$).

thus thought to be brought about by Ca^{2+} influx through a voltage-insensitive pathway as reported previously in an experiment with intracellular Ca^{2+} measurement in cultured aortic smooth muscle (Xuan *et al.*, 1992), since the increased tension was abolished completely by the removal of extracellular Ca^{2+}.

In the carotid arterial smooth muscle from SHRSP, the level of tension, especially the tonic component, attained in the presence of CPA or TG was greater than that in the preparations from WKY. These results are different from those reported in aortic smooth muscle with TG (Tepel *et al.*, 1994). In the present study, the increase in tension of the preparation from SHRSP was greatly reduced in the presence of verapamil, indicating that almost all of the increase in tension by these drugs was mediated by influx of Ca^{2+} through voltage-sensitive channels in this smooth muscle. Since the level of tension attained in the presence of these drugs in the absence of verapamil was greater in the preparations from SHRSP, the amount of Ca^{2+} influx through voltage-sensitive channels may be greater in these preparations. It is also demonstrated in the present experiments that the CPA- or TG-induced tension elevation in the presence of verapamil is smaller in the preparation from SHRSP when compared with that from WKY. This may be explained by smaller voltage-insensitive Ca^{2+} influx in this preparation. The difference in Ca^{2+} accumulation by the sarcoplasmic reticulum may not be involved in this difference, since the contraction remaining in the presence of verapamil was completely abolished by the removal of extracellular Ca^{2+} indicating the involvement of influx but not the release of Ca^{2+} in this increased tension. Differences in the sensitivity of contractile proteins to Ca^{2+} between the two strains can be excluded (Bian & Bukoski, 1995).

Increased Ca^{2+} concentrations were demonstrated in the experiments with the intracellular Ca^{2+} indicator, fura-2. The higher level under non-stimulated conditions in the preparations from SHRSP was in accordance with the increase in basal tone. The result is in agreement with that in aorta which we obtained previously (Sasaki *et al.*, 1993). The higher level of intracellular Ca^{2+} would be brought about by the more depolarized membrane potential in the resting state in the smooth muscle of SHRSP carotid artery as observed in the present experiment. Abolition of the tone by Ca^{2+} removal or by the application of Ca^{2+} antagonist support the possibility of Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The increased level of intracellular Ca^{2+} induced by CPA in the preparations from SHRSP explains the higher level of tension induced by this drug. It was reported that the difference in the TG-induced elevation of the intracellular Ca^{2+} concentration in aortic smooth muscle between preparations from SHR and

WKY was insignificant (Neusser *et al.*, 1993). This discrepancy may have been caused by the degree of hypertension, since they used SHR with mild hypertension and we used SHRSP with severe hypertension.

The effects of verapamil on CPA-induced increase in intracellular Ca^{2+} concentrations agreed with the results of mechanical recording, and indicated that the Ca^{2+} influx mediated through voltage-dependent pathways stimulated by CPA was greater and that through the voltage-independent pathway was smaller, in the preparations from SHRSP. The small increase in intracellular Ca^{2+} concentration in the absence of extracellular Ca^{2+} may have been caused by release from the sarcoplasmic reticulum (Baro & Eisner, 1992; Xuan *et al.*, 1992; Borin *et al.*, 1994). This release of sarcoplasmic reticular Ca^{2+} may be brought about by the net increase due to leakage and the inhibition of Ca^{2+} uptake. However, the amount of the release was too small for the initiation of contraction in this muscle.

The smaller increase in Ca^{2+} concentration in the preparation from SHRSP observed in the presence of verapamil coincides with the smaller contraction observed under the same conditions. The elevation of intracellular Ca^{2+} which causes the increase in tension would be brought about by the influx of extracellular Ca^{2+} as stated above. Again, although CPA or TG may be able to initiate the release of Ca^{2+} from sarcoplasmic reticulum also under these conditions, the amount of released Ca^{2+} would not be sufficient for the increase in tension.

The possibility of the involvement of voltage-dependent Ca^{2+} channels in the increase in tension by CPA was supported by the finding that this drug depolarized the cell membrane of the smooth muscle of the carotid artery in the endothelium-denuded preparations. It has been reported that CPA depolarizes guinea-pig ileal (Uyama *et al.*, 1993) and ureter (Maggi *et al.*, 1995) smooth muscle. This depolarization might be explained by an inhibition of Ca^{2+}-activated K^+ channels (Suzuki *et al.*, 1992; Gagov *et al.*, 1993) or activation of non-specific cation channels (Zhang *et al.*, 1994), and would lead to an increase in opening of voltage-dependent Ca^{2+} channels which are sensitive to verapamil. In addition, these drugs have been reported to activate the Ca^{2+} current through depleting endoplasmic reticular Ca^{2+} (Takemura *et al.*, 1989; Mason *et al.*, 1991; Missiaen *et al.*, 1992; Gericke *et al.*, 1993; Zhang *et al.*, 1994). It is, however, unclear how the membrane permeability to Ca^{2+} is increased in the smooth muscle of rat carotid artery. The depletion of Ca^{2+} from the sarcoplasmic reticulum may activate Ca^{2+} influx as reported in other cell types. The difference in verapamil-sensitive increase in tension between the preparations from WKY and SHRSP may be explained by the difference in the depolarized level of membrane potential. Since it is known that the increase in tension by depolarization is a function of the final membrane potential but not of the degree of depolarization, the more depolarized membrane in the preparation from SHRSP favours activation of the voltage-dependent Ca^{2+} channels, thus leading to greater contraction.

In conclusion, CPA and TG induced contraction of the rat carotid artery. This contraction was associated with an increase in intracellular Ca^{2+} concentration brought about mainly by the increased influx of extracellular Ca^{2+}. Both verapamil-sensitive and -insensitive Ca^{2+} influx are involved in the contraction. The degree of contraction was greater in the preparations from SHRSP than in those from WKY. The greater contraction in the carotid artery of SHRSP was due mainly to the greater influx of Ca^{2+} through voltage-dependent Ca^{2+} channels.

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