Original Article

Ca²⁺ Buffering Function of the Sarcoplasmic Reticulum Is Increased in the Carotid Artery from Spontaneously Hypertensive Rats

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To clarify whether the Ca²⁺ uptake function of the sarcoplasmic reticulum (SR) during arterial contraction is altered in hypertension, the effects of cyclopiazonic acid (CPA) and thapsigargin, which inhibit SR Ca²⁺-ATPase, on the contractile responses to Bay k 8644, an agonist of L-type Ca²⁺ channels, were compared in endothelium-denuded strips of carotid arteries from 13-week-old spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). The addition of Bay k 8644 (1–300 nM) to the strips caused a concentration-dependent contraction that was larger in SHR than in WKY. The contractile responses to Bay k 8644 were augmented by CPA (10 μM) or thapsigargin (100 nM) in both strains. This augmentation was greater in SHR. Each of CPA and thapsigargin induced a relatively transient contraction, and both of these contractions were larger in SHR than in WKY. The basal ⁴⁵Ca influx in this artery was larger in SHR than in WKY. The addition of caffeine (1–20 mM) caused a transient contraction that was larger in SHR than in WKY. Our results indicate that 1) the large Ca²⁺ influx during rest in the SHR carotid artery is strongly buffered by Ca²⁺ uptake into the superficial SR; and 2) the Ca²⁺ uptake function of the SR during the contraction with Bay k 8644 was increased in SHR compared with WKY. We conclude that the SHR carotid artery has an increased total capacity of SR for Ca²⁺ storage as an attempt to compensate for the large Ca²⁺ influx. (*Hypertens Res* 2002; 25: 221–230)

Key Words: sarcoplasmic reticulum, Ca²⁺ buffering function, capacity of SR for Ca²⁺ storage, Ca²⁺ influx via L-type Ca²⁺ channels, SHR carotid artery

Introduction

The sarcoplasmic reticulum (SR) plays an important role in regulating cytosolic Ca²⁺ level ([Ca²⁺]_i) in smooth muscle cells by acting as a buffer barrier to Ca²⁺ influx via L-type Ca²⁺ channels. According to the “superficial buffer barrier” hypothesis (1–3), Ca²⁺ that enters the cells through the plasmalemma is taken up into the superficial (peripheral) SR by SR Ca²⁺-ATPase before it can diffuse to the deeper myo-

plasm (myofilaments). This concept has been confirmed by a number of studies using selective inhibitors of the SR function, such as cyclopiazonic acid (CPA) and thapsigargin, which eliminate the SR Ca²⁺ uptake function through an inhibition of SR Ca²⁺-ATPase (4, 5), and ryanodine, which depletes SR Ca²⁺ through an open-lock of Ca²⁺-induced Ca²⁺ release channels (6–8). Thus, these inhibitors are useful tools for determining the Ca²⁺ buffering function of the SR in arterial smooth muscle (9–13).

In previous studies, we compared the Ca²⁺ buffering func-

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tion of the SR in smooth muscle strips of rat femoral, mesenteric and carotid arteries (12, 13). We reported that the SR of the carotid artery has the following unique characteristics when compared with other arteries: 1) the capacity of the SR to store Ca^{2+} is small as estimated from the contraction induced by caffeine; 2) judging from the effects of CPA and thapsigargin, the Ca^{2+} influx during rest is completely buffered by Ca^{2+} uptake into the SR, but the Ca^{2+} uptake function of the SR during contraction is weak, probably because the SR Ca^{2+} uptake has already reached a near maximum level (*i.e.*, the SR is almost filled to capacity with Ca^{2+}) during rest in this artery; and 3) when the SR is made leaky by ryanodine, Ca^{2+} leaked from the SR during contraction is extruded into the extracellular space.

Spontaneously hypertensive rats (SHR) are used as an experimental model for human essential hypertension (14). We have previously reported that, even in the resting state, the carotid artery of SHR shows greater levels of Ca^{2+} influx and $[\text{Ca}^{2+}]_i$ than the carotid artery of normotensive Wistar-Kyoto rats (WKY) (15, 16). To compensate for the increased level of $[\text{Ca}^{2+}]_i$ in this artery, Ca^{2+} that enters the cells is likely to be taken up into the SR, because the addition of CPA or thapsigargin caused a larger contraction in SHR than in WKY (17). This further suggests that the Ca^{2+} uptake function of the SR during rest in the carotid artery is increased in SHR compared with WKY. However, the Ca^{2+} uptake function of the SR during contraction, the Ca^{2+} movement from the SR and the capacity of the SR to store Ca^{2+} have never been determined in the SHR carotid artery. Therefore, the present study was designed to clarify these SR functions in the SHR carotid artery. The effects of agents that interact with the SR function were determined by measuring the mechanical activity and the ^{45}Ca influx, and the data were compared with those in WKY.

Methods

Preparation of Arterial Strips and Measurement of Isometric Tension

Male SHR and WKY were inbred in our laboratory. They were studied at 13 weeks (12–14 weeks) of age. The systolic blood pressure at this age, measured by tail-cuff plethysmography (KN-210; Natsume Seisakusho, Tokyo, Japan), was significantly higher in SHR (201 ± 1.4 mmHg, $n = 99$) than in WKY (138 ± 0.5 mmHg, $n = 73$). The body weight was not significantly different between SHR (284 ± 1.9 g, $n = 99$) and WKY (289 ± 2.1 g, $n = 73$). After decapitation, common carotid arteries (0.8–1.0 mm outside diameter) were excised and placed in a Krebs solution of the following composition (in mM): NaCl 115.0, KCl 4.7, CaCl_2 2.5, MgCl_2 1.2, NaHCO_3 25.0, KH_2PO_4 1.2 and dextrose 10.0. Arteries were cut into helical strips (0.7–0.8 mm in width and 7–8 mm in length) as described previously (12, 15, 18). To avoid possible influences of endothelium-derived factors, the endotheli-

um of the strip was removed by gently rubbing the endothelial surface with a cotton swab. Successful removal of the endothelium was confirmed by the inability of acetylcholine ($1 \mu\text{M}$) to induce a relaxation in prostaglandin $\text{F}_{2\alpha}$ -precontracted strips.

Arterial strips were mounted vertically in water-jacketed muscle baths containing 10 ml Krebs solution. Krebs solutions were aerated with 95% O_2 and 5% CO_2 and maintained at 37°C . The isometric tension was recorded with a force-displacement transducer (TB-612T; Nihon Kohden Kogyo, Tokyo, Japan). Strips were stretched passively to optimal length by imposing a resting tension of 0.4 g, and a 60-min equilibration period preceded each experiment. All experiments were done under protection from light.

Experimental Protocols

The experimental protocols were similar to those in our previous studies (11–13). After equilibration, contractile responses of the strips to 65.9 mM K^+ Krebs solution (equimolar substitution of Na^+ with K^+) were repeated (usually three times) until the responses were reproducible. After washout with Krebs solution, contractile responses to Bay k 8644 (1–300 nM) or norepinephrine (NE, 10^{-9} – 10^{-4} M) were determined in a cumulative fashion. The NE contraction was determined in the presence of timolol ($0.5 \mu\text{M}$), a β -adrenoceptor antagonist. The sensitivity to Bay k 8644 and NE is expressed as EC_{50} (the concentration inducing 50% of the maximum contraction). To examine the Ca^{2+} buffering function of the SR during the Bay k 8644 or NE contraction, the effects of preincubation with CPA ($10 \mu\text{M}$), thapsigargin (100 nM) or ryanodine ($10 \mu\text{M}$) on these contractions were determined. Because each of these inhibitors induced a relatively transient contraction, the possible mechanism underlying this contraction was also examined. In this experiment, a nominally Ca^{2+} -free solution was used. This solution was prepared by omitting Ca^{2+} from the Krebs solution and by adding EGTA (0.1 mM).

Since the degree of contraction induced by caffeine (20 mM) is a rough index of the amount of Ca^{2+} stored in the SR, this contraction was determined. The degree of NE-induced contraction due to release of SR Ca^{2+} was also determined. Nominally Ca^{2+} -free solution was also used in this experiment.

Measurement of Basal Ca^{2+} Influx

Basal ^{45}Ca influx during rest was measured using a cold La^{3+} wash procedure as described previously (9, 13, 15). Briefly, isolated carotid arteries were opened longitudinally and equilibrated in a Tris-buffered solution of the following composition (in mM): NaCl 154.0, KCl 5.4, CaCl_2 2.5, dextrose 11.0 and Tris 6.0 (pH 7.4). Tris-buffered solutions were aerated with 100% O_2 and maintained at 37°C . Arteries were then placed in the Tris-buffered solution to which ^{45}Ca ($1 \mu\text{Ci/ml}$)

Fig. 1. Typical recordings of the contractions induced by caffeine (Caf; 20 mM), cyclopiazonic acid (CPA; 10 μM), thapsigargin (TG; 100 nM) and ryanodine (Rya; 10 μM) in strips of carotid arteries from SHR. After determination of the maximum contraction induced by 65.9 mM K^+ , Caf was added for 4 min. Following washout for 45 min, CPA (A), TG (B) or Rya (C) was added. In the presence of CPA, TG or Rya, Caf was added. In (C), Caf was again added after washout for 45 min. Dots (•) denote the washout of the strips with Krebs solution.

had been added. After incubation for 1 or 5 min, arteries were placed in a series of four test tubes containing La^{3+} (80.8 mM)-substituted solution (0.5°C) for successive periods of 30 s, 4.5 min, 15 min and 25 min (a total of 45 min) to remove extracellular ^{45}Ca . Arteries were then transferred to a glass scintillation vial containing 0.1 ml Amersham NCS tissue solubilizer (Amersham International, Buckinghamshire, UK). Solubilized tissues were mixed with 5 ml Amersham ACS II scintillant, and their radioactivity was measured in an Aloka liquid scintillation counter. The effects of CPA and ryanodine on the basal ^{45}Ca influx were also determined. Values for basal Ca^{2+} influx were then calculated and are expressed as nmol/g tissue wet weight, as described previously (9, 13, 15).

Statistical Analysis

The results are expressed as the means $\pm$ SEM (n = number of preparations). The Student's t -test for unpaired data was used to determine the significance of differences between means, and a p value of <0.05 was considered to indicate statistical significance.

Drugs and Isotope

The drugs used were CPA (Sigma, St. Louis, USA), thapsigargin (Sigma), ryanodine (Wako, Osaka, Japan), caffeine (Wako), Bay k 8644 (Bayer, Wuppertal, Germany), l -NE bitartrate (Sigma), nifedipine (Bayer Yakuhin, Osaka, Japan), EGTA (Sigma), timolol maleate (Sigma), acetylcholine chloride (Sigma) and prostaglandin $\text{F}_{2\alpha}$ (Ono Pharmaceu-

tical, Osaka, Japan). $^{45}\text{CaCl}_2$ (specific activity initially 20–28.5 mCi/mg) was obtained from Amersham International.

CPA (5 mM) and thapsigargin (100 μM) were dissolved in 100% dimethyl sulfoxide. Bay k 8644 (1 mM) and nifedipine (1 mM) were dissolved in 99.5% ethanol, with further dilution in distilled water before use. Caffeine (1–20 mM) was dissolved in Krebs solution. Aqueous stock solutions were prepared for the other drugs. Concentrations of drugs are expressed as final molar concentrations.

Results

Contractile Responses to CPA, Thapsigargin and Ryanodine

As shown previously (12, 13), each of CPA, thapsigargin and ryanodine induced a relatively transient contraction in strips of carotid arteries from WKY. These experiments were repeated in SHR (Fig. 1). After determination of the 65.9 mM K^+ and caffeine (20 mM) contractions, CPA (10 μM) was added and found to cause a contraction that declined gradually with time (Fig. 1A). The peak contraction induced by CPA was significantly larger in SHR than in WKY (Fig. 2A). After the CPA-induced contraction had declined and reached a plateau (45–55 min), the addition of caffeine caused a transient contraction that was not significantly different from the initial control contraction (Fig. 1A, $n = 7$).

The addition of thapsigargin (100 nM) also caused a contraction that declined gradually with time (Fig. 1B). The peak contraction was again significantly larger in SHR (Fig. 2B). After the thapsigargin-induced contraction had declined

Fig. 2. Contractile responses to cyclopiazonic acid (CPA; 10 μ M, A), thapsigargin (TG; 100 nM, B), ryanodine (Rya; 10 μ M, C) and caffeine (Caf; 20 mM, D) in strips of carotid arteries from SHR and WKY, and effects of Ca^{2+} -free solution (0-Ca) and nifedipine (Nif; 100 nM) on these contractions in SHR. Experimental conditions were the same as in Fig. 1. Control contractions were the total values of the present study. After the 2-min exposure to 0-Ca solution, CPA, TG, Rya or Caf was added. Nif was added 20 min before the addition of CPA, TG or Caf. Contractions induced by CPA, TG, Rya and Caf are expressed as a % of the 65.9 mM K^+ contraction. Data are the means of the number of preparations indicated in parentheses, and SEM are shown by vertical bars. * $p < 0.05$, vs. WKY; # $p < 0.05$, vs. control.

and reached a plateau (70–90 min), the addition of caffeine almost failed to cause a contraction, suggesting that the SR Ca^{2+} was almost depleted during the addition of thapsigargin (Fig. 1B, $n = 7$).

The addition of ryanodine (10 μ M) caused a small contraction (Fig. 1C). This contraction was significantly larger in SHR than in WKY (Fig. 2C). In the presence of ryanodine, the addition of caffeine still caused a transient con-

Fig. 3. Effects of nifedipine (Nif; 100 nM), cyclopiazonic acid (CPA; 10 μ M), ryanodine (Rya; 10 μ M) and 160 mM K^+ (K^+) on basal Ca^{2+} influx in carotid arteries from SHR and WKY. Arteries were incubated for 1 (A) or 5 (B) min in the Tris-buffered solution (5.4 mM K^+) to which ^{45}Ca had been added. Nif was added 20 min before the ^{45}Ca incubation. CPA, Rya or 160 mM K^+ was added to the ^{45}Ca incubation solution. Data are the means of the number of preparations indicated in parentheses, and SEM are shown by vertical bars. * $p < 0.05$, vs. WKY; # $p < 0.05$, vs. control.

traction. After washout, however, caffeine almost failed to induce a contraction, suggesting that the SR Ca^{2+} was almost depleted by this procedure (Fig. 1C, $n = 7$).

Effects of Ca^{2+} -Free Solution and Nifedipine on CPA-, Thapsigargin-, Ryanodine- and Caffeine-Induced Contractions

As shown previously (12), the CPA- and thapsigargin-induced contractions in strips of carotid arteries from WKY were abolished by Ca^{2+} -free solution and were inhibited by nifedipine (100 nM), suggesting that the contractions were due to an influx of extracellular Ca^{2+} . These experiments were repeated in SHR (Fig. 2). The CPA-induced contraction in SHR also was abolished by a 2-min exposure to Ca^{2+} -free solution and was inhibited by nifedipine (Fig. 2A). Similar results were obtained for the thapsigargin-induced contraction (Fig. 2B). The ryanodine-induced contraction was also abolished by Ca^{2+} -free solution (Fig. 2C). On the other hand, the caffeine-induced contraction was not abolished (but was inhibited) by Ca^{2+} -free solution and was not inhibited by nifedipine (Fig. 2D).

Effects of CPA and Ryanodine on Basal Ca^{2+} Influx

We have previously reported that, although both CPA and ryanodine induced a contraction, each agent also decreased the basal Ca^{2+} influx (9, 10, 13). Therefore, these experi-

Fig. 4. Typical recordings of the contractile responses to Bay k 8644 in strips of carotid arteries from SHR. After determination of the maximum contraction induced by 65.9 mM K⁺, contractile responses to Bay k 8644 (Bay; 1–300 nM) were determined in a cumulative fashion (A). Effects of cyclopiazonic acid (CPA; 10 μM), thapsigargin (TG; 100 nM) and the treatment with ryanodine (Rya; 10 μM) plus caffeine (Caf; 20 mM) on the Bay k 8644 contraction are shown in B, C and D, respectively. The incubation of the strips with CPA or TG and the Rya treatment were performed as in Fig. 1. Dots (•) denote the washout of the strips with Krebs solution.

Table 1. Effects of Cyclopiazonic Acid, Thapsigargin and the Ryanodine Treatment on the Contractile Responses to Bay k 8644 (A) or Norepinephrine (B) in Strips of Carotid Arteries from SHR and WKY

Condition	n	Maximum contraction (% of 65.9 mM K ⁺)		EC ₅₀ (nM for Bay; - log M for NE)	
		SHR	WKY	SHR	WKY
A. Bay k 8644 contraction ^a					
Control	9	101.0 ± 5.9 [*]	50.0 ± 5.8	13.5 ± 1.5 [*]	43.0 ± 5.4
+ CPA	9	129.9 ± 5.6 ^{*,#}	82.6 ± 5.9 [#]	5.0 ± 0.7 ^{*,#}	37.3 ± 6.5
+ TG	9	130.7 ± 4.9 ^{*,#}	76.3 ± 7.7 [#]	5.6 ± 0.6 ^{*,#}	47.3 ± 6.3
+ Rya	9	115.1 ± 4.7 [*]	37.8 ± 7.8	18.0 ± 2.7 [*]	80.6 ± 16.8 [#]
B. NE contraction ^b					
Control	10	123.8 ± 5.0	114.7 ± 3.2	8.33 ± 0.09	8.21 ± 0.05
+ CPA	10	133.3 ± 4.3	121.3 ± 4.5	8.59 ± 0.06 ^{*,#}	8.16 ± 0.07
+ Rya	10	124.5 ± 4.2	114.9 ± 3.8	8.03 ± 0.08 ^{*,#}	7.69 ± 0.07 [#]

Data are expressed as the means ± SEM; n, number of preparations. CPA, cyclopiazonic acid (10 μM); TG, thapsigargin (100 nM); Rya, ryanodine (10 μM). ^a Maximum contractions and EC₅₀ values for Bay k 8644 were calculated from the data shown in Fig. 5, and are expressed as a % of the 65.9 mM K⁺ contraction and nM, respectively. ^b Maximum contractions and EC₅₀ values for norepinephrine (NE) were calculated from the data shown in Fig. 6, and are expressed as a % of the 65.9 mM K⁺ contraction and a negative log of the molar concentration (- log M), respectively. *p < 0.05, vs. WKY; #p < 0.05, vs. control.

ments were performed in the carotid arteries from SHR and WKY. The basal Ca²⁺ influx in the arteries measured by an incubation with ⁴⁵Ca for either 1 or 5 min was significantly larger in SHR than in WKY (Fig. 3). The basal Ca²⁺ influx

was significantly decreased by nifedipine (100 nM) in SHR but not in WKY (Fig. 3). When the arteries were incubated for 5 min with ⁴⁵Ca in the presence of CPA (10 μM), the influx was significantly decreased in both strains (Fig. 3B).

Fig. 5. Effects of cyclopiazonic acid (CPA; 10 μ M), thapsigargin (TG; 100 nM) and the ryanodine (Rya; 10 μ M) treatment on the concentration-response curve for Bay k 8644 in strips of carotid arteries from SHR (upper panel) and WKY (lower panel). Experimental conditions were the same as in Fig. 4. At "0" on the abscissa, the CPA-, TG- and Rya-induced contractions remaining before the addition of Bay k 8644 are expressed as a % of the 65.9 mM K⁺ contraction. The peak contractions induced by each concentration of Bay k 8644 are then expressed as a % of the 65.9 mM K⁺ contraction. Data points are the means of 9 preparations, and SEM are shown by vertical bars. * $p < 0.05$, vs. control.

Similar results were obtained for ryanodine (10 μ M) (Fig. 3B). In contrast, the influx was significantly increased by 160 mM K⁺ solution in both strains (Fig. 3B). This increase was significantly larger in SHR (Fig. 3B).

Effects of CPA and Thapsigargin on the Contractile Responses to Bay k 8644

The addition of Bay k 8644 (1–300 nM) to the strips caused a concentration-dependent rhythmic contraction in both SHR and WKY (Figs. 4A and 5). The maximum contraction induced by Bay k 8644 was significantly larger in SHR and the EC₅₀ value for Bay k 8644 was significantly lower in SHR (Table 1A).

The difference in Bay k 8644 contraction between the two strains reflects the possibility that, if the Ca²⁺ uptake function of the SR is weak in SHR, a large portion of the Ca²⁺ that enters the cells during Bay k 8644 stimulation bypasses the SR to reach the myofilaments, which would account for the strong contraction seen here. To test this possibility, the

Fig. 6. Effects of cyclopiazonic acid (CPA; 10 μ M) and the ryanodine (Rya; 10 μ M) treatment on the concentration-response curve for norepinephrine (NE) in strips of carotid arteries from SHR (upper panel) and WKY (lower panel). Experimental conditions were the same as in Figs. 4 and 5, except that NE (10^{-9} – 10^{-4} M) was used as a contractile agonist. At "0" on the abscissa, the CPA- and Rya-induced contractions remaining before the addition of NE are expressed as a % of the 65.9 mM K⁺ contraction. The peak contractions induced by each concentration of NE are then expressed as a % of the 65.9 mM K⁺ contraction. Data points are the means of 10 preparations, and SEM are shown by vertical bars. * $p < 0.05$, vs. control.

effects of CPA (10 μ M) and thapsigargin (100 nM) on the Bay k 8644 contraction were compared between the two strains. After the CPA-induced contraction had declined and reached a plateau, Bay k 8644 was added (Figs. 4B and 5). Under these conditions, the Bay k 8644 contraction was augmented in both strains. Judging from the augmented area under the concentration-response curves, the augmentation was significantly greater in SHR (Fig. 5, Table 1A). Similar results were obtained when the effect of thapsigargin (100 nM) was compared between the two strains (Fig. 5, Table 1A). Thus, the Ca²⁺ uptake function of the SR during the Bay k 8644 contraction was significantly greater in SHR than in WKY.

Effects of CPA on the Contractile Responses to NE

The addition of NE (10^{-9} – 10^{-4} M) to the strips caused a

Fig. 7. Contractile responses to caffeine (Caf) in strips of carotid arteries from SHR and WKY. (A) Typical recordings of the caffeine contractions in SHR. The contractile responses to caffeine (1–20 mM) were determined in a non-cumulative fashion. (B) Summary of the caffeine contractions in SHR and WKY. The peak contractions induced by each concentration of caffeine are expressed as a % of the 65.9 mM K^+ contraction. Data points are the means of 6 preparations, and SEM are shown by vertical bars. * $p < 0.05$, vs. WKY.

concentration-dependent contraction in both SHR and WKY (Fig. 6). The contraction induced by 10^{-9} M NE was significantly larger in SHR than in WKY (Fig. 6). However, the maximum contraction and the EC_{50} value for NE were not significantly different between the two strains (Table 1B).

The effects of CPA (10 μM) on the NE contraction were compared between the two strains (Fig. 6, Table 1B). In the presence of CPA, the NE contraction (especially the contraction induced by 10^{-9} M NE) was augmented in SHR, while in WKY, the contraction was unchanged (Fig. 6, Table 1B). Again, the Ca^{2+} uptake function of the SR during the NE contraction was significantly greater in SHR than in WKY.

Effects of Ryanodine on the Contractile Responses to Bay k 8644 and NE

The effect of ryanodine on the Bay k 8644 contraction was compared between SHR and WKY (Figs. 4D and 5, Table 1A). After the treatment with ryanodine (10 μM) plus caffeine (20 mM), Bay k 8644 was added (Figs. 4D and 5). Under these conditions, the Bay k 8644 contraction was unchanged in SHR but was reduced in WKY (Fig. 5, Table 1A). After the ryanodine treatment, the pattern of the Bay k 8644 contraction was different from that under other conditions (Fig. 4).

Fig. 8. Contractile responses to norepinephrine (NE) determined in Ca^{2+} -free solution (0-Ca) in strips of carotid arteries from SHR and WKY. (A) Typical recordings of the NE contractions in 0-Ca solution (, 2-min exposure) in SHR. The contractile responses to NE (10^{-9} – 10^{-5} M) were determined in a non-cumulative fashion. (B) Summary of the NE contractions in 0-Ca solution in SHR and WKY. The peak contractions induced by each concentration of NE are expressed as a % of the 65.9 mM K^+ contraction. Data points are the means of 6 preparations, and SEM are shown by vertical bars. * $p < 0.05$, vs. WKY.

The effect of ryanodine on the NE contraction was then compared between the two strains (Fig. 6, Table 1B). After the ryanodine treatment, the NE contraction was reduced in both strains. Judging from the reduced area under the concentration-response curves, the reduction was significantly less in SHR (Fig. 6, Table 1B).

Contractile Responses to Caffeine

The addition of caffeine (1–20 mM) to the strips caused a concentration-dependent transient contraction in both SHR and WKY (Fig. 7). The contraction induced by each concentration of caffeine was significantly larger in SHR (Fig. 7). The EC_{50} value for caffeine to induce a contraction was significantly lower in SHR (2.4 ± 0.4 mM, $n = 6$) than in WKY (4.1 ± 0.6 mM, $n = 6$).

Contractile Responses to NE in Ca^{2+} -Free Solution or in the Presence of Nifedipine

The addition of NE (10^{-9} – 10^{-5} M) to the strips placed in Ca^{2+} -free solution caused a concentration-dependent transient contraction in both SHR and WKY (Fig. 8). The contractions induced by high concentrations of NE (10^{-6} and

Fig. 9. Effects of nifedipine (Nif; 100 nM) on the concentration-response curve for norepinephrine (NE) in strips of carotid arteries from SHR and WKY. Experimental conditions were similar to those in Fig. 6. Nif was added 20 min before the determination of the concentration-response curve for NE. The peak contractions induced by each concentration of NE are expressed as a % of the 65.9 mM K^+ contraction. Data points are the means of 6 preparations, and SEM are shown by vertical bars. * $p < 0.05$, vs. WKY.

10^{-5} M) were significantly larger in SHR (Fig. 8).

The contraction induced by a low concentration of NE (10^{-9} M) in both strains (Fig. 6) was not due to the SR Ca^{2+} , because this contraction was almost abolished by Ca^{2+} -free solution (Fig. 8). This contraction was dependent on Ca^{2+} influx via L-type Ca^{2+} channels, because it was almost abolished by 100 nM nifedipine in both strains (Fig. 9). Judging from the reduced area under the concentration-response curves, the total reduction of the NE contraction by nifedipine was significantly greater in SHR than in WKY (Fig. 9).

Discussion

In the present study, we examined the Ca^{2+} buffering function of the SR in strips of carotid arteries from SHR and WKY by measuring the arterial contractions after administration of selective agents of SR function. We conclude that the SHR carotid artery has an increased total capacity of SR for purpose of Ca^{2+} storage to compensate for the large Ca^{2+} influx during both rest and contraction.

That the Ca^{2+} uptake of the SR was increased during rest in the SHR carotid artery is supported by the following observations: 1) when the SR Ca^{2+} uptake function was eliminated by CPA or thapsigargin, there was a larger contraction in SHR than in WKY; and 2) the basal Ca^{2+} influx measured by a 1- or 5-min incubation with ^{45}Ca was larger in SHR than in WKY. We previously reported that, even in the resting state, the carotid artery of SHR shows a larger Ca^{2+} influx and higher level of $[Ca^{2+}]_i$ than the carotid artery of

WKY (15, 16). The large Ca^{2+} influx in this artery was thus further confirmed in the present study. We predicted that, if the SR of the SHR carotid artery is an effective barrier to the high basal $[Ca^{2+}]_i$, elimination of the SR Ca^{2+} uptake by CPA or thapsigargin would cause a large elevation of $[Ca^{2+}]_i$ and a strong contraction.

The results that the contractile responses to CPA and thapsigargin were inhibited by nifedipine may suggest that L-type Ca^{2+} channels are directly activated by these agents. Although such a direct action has never been reported, Sekiguchi *et al.* (17) reported that CPA depolarized the smooth muscle membrane of the carotid arteries, probably due to an inhibition of Ca^{2+} -activated K^+ channels via the depletion of SR Ca^{2+} and/or an activation of nonspecific cation channels. However, in the present study, the following findings provided no support for the idea that CPA has a membrane depolarizing effect: 1) after a 5-min incubation with ^{45}Ca , CPA decreased the basal Ca^{2+} influx; and 2) SR Ca^{2+} was not depleted during the addition of CPA. We consider that the contractile response to CPA or thapsigargin reflects the extent of the Ca^{2+} uptake function of the SR against the basal Ca^{2+} influx, as discussed previously (10, 12, 19, 20). Thus, upon elimination of Ca^{2+} uptake into the SR by CPA or thapsigargin, a large portion of the Ca^{2+} that enters the cells during rest bypasses the SR and reaches the myofilaments, resulting in a contraction. It also is possible that a fraction of the Ca^{2+} that enters the cells is extruded into the extracellular space, as shown previously (10, 20).

That the uptake of Ca^{2+} by the SR is increased during contraction in the SHR carotid artery is supported by the following observations: 1) the extent of the augmentation by CPA or thapsigargin of the Bay k 8644 contraction was greater in SHR than in WKY; and 2) the augmentation by CPA of the NE contraction was observed only in SHR. Thus, upon elimination of SR Ca^{2+} uptake by CPA or thapsigargin, a large portion of the Ca^{2+} that enters the cells during the stimulation with Bay k 8644 or NE bypasses the SR and reaches the myofilaments, resulting in the observed augmentation of contraction.

The Bay k 8644 contraction was larger in SHR than in WKY. An increased Bay k 8644 contraction has previously been reported in SHR arteries (21, 22). The larger contractions in SHR can be easily explained by the assumption that the Ca^{2+} uptake function of the SR is weak during the Bay k 8644 stimulation. However, this is unlikely, because the elimination of SR Ca^{2+} uptake by CPA or thapsigargin caused a greater augmentation of the Bay k 8644 contraction, as described above. Therefore, during the Bay k 8644 stimulation, there is a large Ca^{2+} influx that exceeds the Ca^{2+} uptake function of the SR, and this influx may be larger in SHR than in WKY. This assumption is supported by the result (Fig. 3B) that during the 160 mM K^+ stimulation the Ca^{2+} influx was larger in SHR than in WKY.

There have been two studies comparing the augmenting effects of CPA on the Bay k 8644 or K^+ contraction between

SHR and WKY (19, 23). Low *et al.* (23) reported that addition of 30 μM CPA to strips of the aorta caused a sustained contraction in both strains, and this contraction was larger in SHR. CPA also augmented the K⁺ contraction in both strains, and this augmentation was greater in SHR. These observations were similar to those for the carotid arteries in the present study. On the other hand, Nomura and Asano (19) reported that addition of 10 μM CPA to strips of the tail artery caused a similar transient contraction in both strains. CPA also augmented the Bay k 8644 or K⁺ contraction to a similar extent in both strains. These observations are different from those of the present study. The difference between the observations in the aorta and the carotid artery (both of which showed greater effects in SHR) and those in the tail artery (similar effects for SHR and WKY) may be due to the difference in the total capacity of SR for Ca²⁺ storage among these arteries; this capacity is larger in the aorta and the carotid artery from SHR than in the same arteries in WKY (24, the present study), but is the same for the tail arteries from SHR and WKY (19). Thus, the total capacity of SR for Ca²⁺ storage is an important factor for determining the Ca²⁺ buffering function of the SR in arterial smooth muscles.

In previous studies, the carotid arteries have been shown to be more sensitive to NE (low EC₅₀ values) than other arteries (12, 13). In the present study, the contractile response to 10⁻⁹ M NE was larger in SHR, and the responses to medium to high concentrations of NE were almost the same for the two strains. Interestingly, the contraction induced by 10⁻⁹ M NE in SHR was highly augmented by CPA. This contraction was due to Ca²⁺ influx via L-type Ca²⁺ channels, since it was abolished by either nifedipine or Ca²⁺-free solution. Thus, the effects of either CPA or thapsigargin on the responses to Bay k 8644 can be expected to be similar to those on the responses to 10⁻⁹ M NE.

The Ca²⁺ movement from leaky SR was also different between SHR and WKY. As discussed previously (13), under the establishment of a leaky SR by ryanodine, the stimulation with K⁺ or NE causes an influx of Ca²⁺ via L-type Ca²⁺ channels, and a fraction of the entering Ca²⁺ is initially taken up into the leaky SR before it reaches the myofilaments, where it can move to several regions of the cytoplasm. When Ca²⁺ moves to the myofilaments, the contraction should be augmented. In contrast, when Ca²⁺ moves to the plasmalemma, the extrusion of Ca²⁺ from the cells by the Na⁺-Ca²⁺ exchanger and the Ca²⁺-ATPase of the plasmalemma and/or the inhibition of Ca²⁺ influx through the activation of Ca²⁺-activated K⁺ channels (*i.e.*, Ca²⁺ sparks) should occur, resulting in an inhibition of the contraction. It is thus likely that, in the case of a leaky SR, Ca²⁺ leaked from the SR moves to both the myofilaments and the plasmalemma, such that the final response to K⁺ or NE could be determined by the net balance of these two events. In the present study, the WKY artery with a leaky SR showed reduced Bay k 8644 and NE contractions, suggesting that Ca²⁺ moved mainly to the plasmalemma rather than to the myofilaments, in agree-

ment with the previous study (13). In the SHR artery with a leaky SR, the Bay k 8644 contraction was not altered and the NE contraction was slightly reduced, indicating that in addition to the Ca²⁺ movement toward the plasmalemma as in WKY, a fraction of the Ca²⁺ leaked from the SR reached the myofilaments. These observations are unique to the rat carotid arteries: in other rat arteries with a leaky SR, such as the femoral, mesenteric and tail arteries, the contractile responses to Bay k 8644, K⁺ and NE have all been shown to be augmented, suggesting that the Ca²⁺ moves mainly to the myofilaments rather than to the plasmalemma (11, 13, 19). Furthermore, the net balance of the two events may contribute to the pattern of the Bay k 8644 contraction after the ryanodine treatment shown in Fig. 4D.

The contraction induced by caffeine or NE in Ca²⁺-free solution was larger in SHR than in WKY. A large caffeine contraction has also been reported in other arteries of SHR (9, 24, 25). According to the "superficial buffer barrier" hypothesis (1-3), the effectiveness of the SR at buffering Ca²⁺ depends on the state of Ca²⁺ loading of the SR; an empty SR will buffer all stimulated Ca²⁺ influx and a filled SR cannot buffer the entering Ca²⁺. In the present study, an increased Ca²⁺ uptake function of the SR in the SHR carotid artery was observed not only during rest but also during the contraction with Bay k 8644. Thus, the SHR carotid artery has an increased total capacity of SR for Ca²⁺ storage when compared with WKY.

In the present study, the contractile responses to CPA, thapsigargin, ryanodine, caffeine, Bay k 8644 and NE were all larger in SHR than in WKY. The larger contractions in the SHR artery may reflect the increased Ca²⁺ sensitivity of myofilaments in this artery. However, this is unlikely, because the Ca²⁺ sensitivity of myofilaments in the SHR artery has been reported to be similar to that in WKY (26-29).

Altogether, the results of the present study suggest that 1) the large Ca²⁺ influx during rest in the SHR carotid artery is strongly buffered by Ca²⁺ uptake into the superficial SR; and 2) the Ca²⁺ buffering function of the SR during the contraction with Bay k 8644 or NE is higher in SHR than in WKY. We conclude that, during both rest and contraction, the SHR carotid artery increases its total SR capacity in order to store Ca²⁺ and thereby offset the large Ca²⁺ influx.

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